Proceedings of the 31st Annual Aquatic Toxicity Workshop: October 24 to 27, 2004, Charlottetown, Prince Edward Island

Comptes rendus du 31éme atelier annuel sur la toxicité aquatique: du 24 au 27 october 2004, Charlottetown, île du Prince Édouard

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2004

Canadian Technical Report of Fisheries and Aquatic Sciences 2562

Rapport technique canadien des sciences halieutiques et aquatiques 2562

Fisheries and Oceans Canada
Pêches et Océans Canada

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Cat. No. Fs 97-6/2562
ISSN 0706-6457

Citation for this publication/On devra citer la publication comme suit:

Burridge, L.E., K. Haya and A.J. Niimi (eds). 2004. Proceedings of the 31st Annual Aquatic Toxicity Workshop: October 24-27, 2004, Charlottetown, Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci.: 2562. 138 p.

Burridge, L.E., K. Haya et A.J. Niimi (eds). 2004. Comptes rendus du 31éme atelier annuel sur la toxicité aquatique: du 24 au 27 octobre 2004, Charlottetown, île du Prince Édouard. Rapp. tech. can. sci. halieut. aquat.: 2562. 138 p.

Preface / Preface

The 31st Annual Aquatic Toxicity Workshop was held at the Delta Prince Edward in Ottawa, Ontario, Charlottetown, Prince Edward Island, October 24-27, 2004. The Workshop included 4 plenary presentations, 119 platform and 77 poster papers. Total attendance was 299.

This Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are administered by a Board of Directors, and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 31éme atelier annuel sur la toxicité a eu lieu Delta Prince Édouard, Charlottetown, île du Prince Édouard, du 28 au 27 october 2004. Le atelier a donné lieu a 4 communication lors de séances plénières, 119 exposés d'invités d'honneur 77 communications par affichage. 299 personnes ont assisté au atelier.

Le atelier a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

Editors comments / Remarques des editeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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Table of Contents/Tables des matiére

Plenary/Plénière

Page

A vision for aquatic toxicology in the Department of Fisheries and Oceans. S. Paradis. 1

Agricultural practices in Prince Edward Island for a sustainable environment. R.M. Cheverie. 1

Water on Prince Edward Island: precious resource or abused commodity? D.L. Guignion. 1

National Agri-Environmental Standards Initiative for the Agricultural Policy Framework. J.L. Papineau and J. Jarjour. 2

Contributed Papers/Documents contribués

Environmental Effects Monitoring - pulp and paper/Étude de suivi des effets sur l'environnement - pâtes et papiers 3

Overview of cycle 3 findings of aquatic effects due to pulp and paper mill effluents. B.L. Ring, K. Hedley, R.B. Lowell, G. Pastershank, L. Trudel and S.L. Walker. (PL)* 3

Pulp and paper mill effluent effects on invertebrates: national response patterns over two Environmental Effects Monitoring cycles. R.B. Lowell, B.L. Ring, G. Pastershank, L. Trudel and K. Hedley. (PL) 3

National assessment of pulp and paper mill effluents on fish: comparison between two Environmental Effects Monitoring cycles. L. Trudel, G. Pastershank, R.B. Lowell, B.L. Ring and K. Hedley. (PL) 4

Fish'n with variability in the Athabasca River. R. Shelast, M. Luoma and M. Spafford. (PL) 4

Patterns of benthic diversity and nutrient enrichment in a tidal river receiving environment for a pulp and paper mill in New Brunswick. M.H. Murdoch, M. Stephenson, A. Gallant and J.-Y. Ritchie. (PL) 4

Comparing apples to orchards: taxonomic resolution and Environmental Effects Monitoring marine benthos effects assessment. L. Uhlig, M. Davies, M. Ptashynski and W.N. Gibbons. (PL) 5

Investigation of cause in pulp and paper Environmental Effects Monitoring. L.M. Hewitt, M.G. Dubé, S.C. Ribey, J.M. Culp, B.W. Kilgour, C.B. Portt, K. Hedley, D.L. MacLatchy and K.R. Munkittrick. (PL) 7

Use of fathead minnow (*Pimephales promelas*) in assessing reproductive effects of pulp mill effluent for investigation of cause studies. C.J. Rickwood, M.G. Dubé, D.L. MacLatchy, L.M. Hewitt and J.L. Parrott. (PL) 8

Statistical analysis tool (SAT) for Environmental Effects Monitoring analysis – how cool is this? S.C. Ribey, M.G. Dubé, R.B. Lowell, G.R. Champagne, I. Wong and J. Inkster. (PL) 8

---

*PL: Platform paper. PO: Poster paper.

Pulp and paper mill effluent nutrient contributions to receiving waters in relation to biological effects at a watershed scale. T. Hall and W. Arthurs. (PL) 9
A decade later: intertidal diversity is increased as pulp mill pollution is decreased. S.M. Bard, F. De Raedemaecker and W. Willems. (PL) 9
Proteomic profiling of teleost cell lines for high throughput aquatic toxicity assessment. S.K. Wagg and L.E.J. Lee. (PO) 9
Nutrient - algal accrual dose response curves using pulp mill and municipal effluents: an investigation of cause scenario on the Wapiti River, Alberta, Canada. N.E. Glozier, J.M. Culp, K.J. Cash, B.K. Firth and G. Wilson. (PO) 10
Environmental Effects Monitoring Cycle 3 fish survey results for pulp and paper mills discharging to Lake Superior, Ontario. N. Ali, D. Audet and K. Flood. (PO) 10

Pesticides /Pesticides

Concentrations of pesticides in water samples from St. Lawrence Estuary tributaries. M. Lebeuf, C.M. Couillard, R.L. Roy, C. DeBlois, S. Trottier, M. Noël, A. Ouellet and G. Allard. (PL) 11
Toxicity of pesticides in short-term. P.M. Jackman and K.G. Doe. (PL) 11
Does pesticide use in Prince Edward Island potato farms cause estrogenic responses in fish? J.P. Sherry, C. Tinson, K. Cooper, R. Mroz, W.R. Ernst, M.R. Servos and L. Vallis. (PL) 12
Use of confocal laser scanning microscopy for the study of vitellogenesis in Toxaphene exposed female yellowtail flounder (*Limanda ferruginea* Storer). G.E. Fåhræus Van Ree. (PL) 12
Depression of brain acetylcholinesterase (AChE) in fish collected downstream of agricultural activities. M.A. Gray, C.J. Smith, B.J. Park, V.P. Palace and K.A. Kidd. (PL) 13
Examinations of the potential for pesticides to impact golden shiners in a Niagara stream. B.J. Park, J. Struger, J.L. Parrott, R.E. Evans, K.G Wautier, C.L. Baron, K.A. Kidd, M.A. Gray and V.P. Palace. (PL) 13
Pesticide science fund project - reducing pesticide impacts in aquatic systems of the Atlantic region. W.R. Ernst, A. Denning, H. Rees, L. Chow, J.M. Culp, K.G. Doe, L.M. Hewitt, G. Julien, C. Murphy and J. Hellou. (PL) 14
Pesticides, fish and fish habitat: a greater evaluation of risks is required. J.F. Payne. (PL) 14
Multi component pesticide studies in the St. Lawrence River valley. L. Poissant, F. Aulagnier, C.Beauvais, J. Boyer, M. Garmouma and M. Pilote. (PL) 15
Reducing pesticide environmental risks: The National Agri-environmental Standards Initiative (NAESI). P.-Y. Caux and P.B. Jiapizian. (PL) 15
Concentrations of pesticides in critical zones of the Saint Lawrence Estuary and their toxicity to fish. C.M. Couillard, M. Lebeuf, R.L. Roy and C. DeBlois. (PO) 15
Residues of organochlorine pesticides in farm areas of the lower Fraser Valley, British Columbia, Canada. J.-N. Kuo, M.T. Wan and J. Pasternak. (PO) 16
Preliminary analyses of organochlorine pesticide residues in sport fish from Lake Erie. G. Tomasino, R. Grebenok and P.F. Dehn. (PO) 16

An assessment of the effectiveness of 10 m buffer zones in minimizing pesticide runoff from potato fields in Prince Edward Island – 2003 results. A. Denning, A. Cook, K.G. Doe, W.R. Ernst and G. Julien. (PO) 17

Pesticides in water and surface sediments of an estuary on Prince Edward Island, Canada. J. Hellou, A. Cook, W.R. Ernst, J. Leonard and S. Steller. (PO) 17

How to improve the environmental realism of aquatic pond mesocosms for regulatory decisions on pesticide registration? E.M. Foekema. (PO) 21

Effect of exposure duration and dissolved oxygen condition on chronic imidacloprid toxicity to *Chironomus tentans*. S.J. Stoughton, K. Liber, J.M. Culp and A. Cessna. (PO) 21

Effects of the insecticide imidacloprid on *Epeorus longimanus* and *Lumbriculus variegatus* feeding and growth. A. Alexander, J.M. Culp and D. Baird. (PO) 21

Ecological risk assessment / Évaluation des risques écologiques

Reducing uncertainty in Environmental Impact Assessment (EIA) by integration of Environmental Effect Monitoring (EEM) and Environmental Risk Assessment (ERA). M. Smit, C. Karman and J. van Dalfsen. (PL) 22

Null variables in environmental monitoring. M.D. Paine. (PL) 23

Receiving environment monitoring program for Lions Gate Wastewater Treatment Plant, Burrard Inlet, BC: a program under development. F. Bishay, S.D. St-Jean and P. van Poppelen. (PL) 23

Development of a Greater Vancouver Regional District trigger process for the protection of the receiving environment. A. van Roodselaar, B. Burd, A. Lewis, P. van Poppelen, F. Bishay and S. Bertold. (PL) 24

Strategy for the categorization of polymeric substances on Canada's Domestic Substances List. A. Séné, D. Dubé and N. Davidson. (PL) 31

Ecotoxicity testing framework for petroleum hydrocarbon-contaminated site soils as part of a Tier 2 site-specific assessment. G.L. Stephenson and N.C. Feisthauer. (PL) 31

Deterministic and probabilistic risk assessments of pharmaceuticals and personal care products in wastewater treatment plants effluents based on the lowest toxicity values. K.D. Bergh and F.C.P. Law. (PL) 31

The application of the Canadian Council of Ministers of the Environment (CCME) Water Quality Index (WQI) as a screening tool to evaluate the suitability of waters for aquaculture. R. Paterson, H. Khan, R. Crewe and A.A. Khan. (PL) 32

Ecological risks due to current PAH loading from Sydney Tar Ponds to Sydney Harbour. D.W. Smith, W. Van Veen and B. McRoberts. (PL) 32

A decision-making framework for contaminated sediments. P.M. Chapman and J. Anderson. (PL) 33

Draft environmental screening assessment of polybrominated diphenyl ethers in Canada. J.P. Pasternak, L. Suffredine and K. Taylor. (PO) 33

Categorizing the Domestic Substances List: update on "Unknown or Variable composition, Complex reaction products and Biological materials" (UVCBs). S. Schnabel, A. Okonski, A. Séné and J. Gauthier. (PO) 34

Assessing ecotoxicity studies submitted under the *New Substances Notification Regulations* in Canada: scoring method and regulatory uses. M.J. Lapointe, A. 34

Pigeon, M. Lortie, M. Lewis, A.J. Atkinson, R.L. Breton, R. Thompson and G. Gilron. (PO)

Development of a surface water quality objective for uranium for application in northern Saskatchewan. T.S. Moulding. (PO) 35

Challenges in amphibian and reptile risk assessment and possible paths forward. L.J. Marshall, R.D. Willis and C.E. Moore. (PO) 35

An update on Environment Canada's screening assessment activities for perfluoroalkyl compounds. A. Miettunen. (PO) 35

Amending environmental regulations to protect aquatic ecosystems from biological pollutants. A.J. Niimi. (PO) 36

Microbial source tracking – identifying non-point sources of fecal pollution. G.C. van Aggelen and H. Osachoff. (PO) 36

A relative risk ranking of the National Pollutant Release Inventory (NPRI). A.M. Dunn. (PO) 37

The relationship between sediment type and benthic macroinvertebrates mediated through aquatic macrophyte development: experimental sediment exchanges between natural and constructed wetlands on oil sands leases, near Fort McMurray, Alberta. L. Barr, N. Cooper, L. Foote, W. Tedder and J.J.H. Ciborowski. (PO) 37

Toxicants in surface waters in two Nigerian coastal communities. G.R.E.E. Ana, M.K.C. Sridhar and G.O. Emerole. (PO) 38

Sediment disposal at sea / Immersion en mer des sédiments

Current research and monitoring of Environment Canada's disposal at sea program. P.A. Topping. (PL) 38

Integrated techniques for monitoring of an offshore disposal site in a dynamic tidal environment. R.D. Parrott, M.B. Parsons, M.Z. Li, V.E. Kostylev, J.E.H. Clarke, A. Duxfield and K.-L. Tay. (PL) 39

Environmental monitoring of dredged material ocean disposal sites in Atlantic region. K.-L. Tay, K.G. Doe, A. MacDonald, R. Mroz, K. Lee and R.D. Parrott. (PL) 39

A survey of tributyltin residues at selected ocean disposal sites and harbours in Canada. J.A. Thompson, P.A. Topping and R.C.H. Wilson. (PL) 40

Sediment chemistry and toxicity at the Point Grey disposal site. R.C.H. Wilson, S. McKinnon and D. Sullivan. (PL) 40

Sand and surf – a new approach to managing Fraser River dredge spoils. S. Standing and P. Hill. (PL) 41

Pacific and Yukon Region disposal at sea website. J.E. Wilkinson. (PL) 41

Disposal at sea marine sediment guideline for cadmium: review and recommendations for the British Columbia coast. C.D. Campbell, R. Leung and D.L. Sullivan. (PO) 41

Wood waste associated with dredged materials: potential management options. R. Leung and S. Standing. (PO) 42

Sand Heads ocean disposal site: managing risk. S. Standing and R. Leung. (PO) 42

Wood waste management initiative. J.E. Wilkinson and S. Standing. (PO) 42

Oil and gas / Huiles et graisses

Terra Nova Environmental Effects Monitoring program: from environmental impact statement onward. E. DeBlois, M.D. Paine, F. Power and U. Williams. (PL) 43

Hibernia Environmental Effects Monitoring programs: sediment chemistry profiles and their implications for environmental impacts. S.A. Whiteway and R.A. Dunphy. (PL) 43

Hibernia Environmental Effects Monitoring programs: sediment toxicity data 1994-2004. S.A. Whiteway and R.A. Dunphy. (PL) 43

Effective monitoring programs for oil development: the program for the Grand Banks of Newfoundland. J.F. Payne. (PL)

Effect of salinity on the uptake and toxicity of PAH from chemically dispersed oil. S.D. Ramachandran, M. Boudreau, S.C. Courteney, T. King, J.A. Dixon, M.J. Sweezey and P.V. Hodson. (PL) 44

Toxicity of crude oil to early life stages of two fish species. L.M. Clarke, R.S. Brown, T. King, K. Lee and P.V. Hodson. (PL) 45

Assessment of freshwater sediment toxicity from enhanced anaerobic vegetable oil degradation. K. Lee, G. Wohlgeschaffen, T. King, S.E. Cobanli, K.G. Doe, P.M. Jackman, B.A. Wrenn, Z. Li and A. Venosa. (PL) 45

Identification of CYP1A inducing compounds in crude oil. C.W. Khan, B.P. Hollebone, Z. Wang, R.S. Brown and P.V. Hodson. (PL) 46

Isolation of CYP1A inducing components in coal tar fraction (F3) of Alaska north slope crude oil – a preliminary study. G. Saravanabhavan, C.W. Khan, R.S. Brown and P.V. Hodson. (PL) 46

Use of polyethylene membrane devices for monitoring diesel oil contamination on and within beaches. J.W. Short, S.D. Rice, M.R. Lindeberg and M.G. Carls. (PL) 47

Development of water quality objectives and management systems for the lower Athabasca River in the oil sands area. L. Noton and P. McEachern. (PL) 53

Ecotoxicological assessment of using oil sands coke in aquatic reclamation strategies. A.J. Squires and K. Liber. (PL) 54

Using stable carbon isotopes to monitor bacterial degradation of naphthenic acids and polycyclic aromatic hydrocarbons. P.P. Videla, A.J. Farwell, B.J. Butler, V. Nero and D.G. Dixon. (PL) 54

Microbial carbon sources in boreal wetlands. C.A. Daly and J.J.H. Ciborowski. (PO) 55

The role of stress in ecosytem properties: the benthic invertebrate community of the Alberta oil-sands wetlands. C.M. Wytrykush and J.J.H. Ciborowski. (PO) 55

Histopathological effects of naphthenic acids and salinity to yellow perch, *Perca flavescens*. V. Nero, A.P. Farwell, L.E.J. Lee and D.G. Dixon. (PO) 56

The use of stable carbon isotopes to examine the degradation of naphthenic acids. A.P. Farwell, B.J. Butler, V. Nero, P.P. Videla and D.G. Dixon. (PO) 56

Rapid toxicological evaluation of oil-sands' process-affected water using fish cell lines. V.R. Dayeh, V. Nero, A.P. Farwell, D.G. Dixon, N.C. Bols and L.E.J. Lee. (PO) 56

Chronic toxicity study on snow crab exposed to drilling fluid being used on the Grand Banks. C.D. Andrews, B. French, L. Fancy, J. Guiney and J.F. Payne. (PO) 57

Pilot study on histopathological abnormalities in winter flounder in association with 57

Sydney tar ponds. A. Mathieu, R. Soper and B. French. (PO)
The environmental impact factor; a risk assessment tool for the offshore oil and gas industry validated and calibrated with effect monitoring data. M. Smit, C. Karman and R. Jak. (PO) 58
Polycyclic aromatic hydrocarbons in Northwest Atlantic finfish: available and needed knowledge for monitoring. J. Hellou, J. Leonard, T.K. Collier and F. Ariese. (PO) 58
The toxicity of photomodified polycyclic aromatic hydrocarbons and dibenzothiophene congeners to Japanese medaka embryos. A.P. Farwell, P.S. Bal, M. Croft and D.G. Dixon. (PO) 61
Effects of petroleum coke on development of invertebrate and macrophyte communities in constructed wetlands. L.F. Baker, J.J.H. Ciborowski and M.D. MacKinnon. (PO) 61

Pharmaceuticals/Produits pharmaceutiques

Partitioning and biodegradation: determining the fate of phenolic estrogenic compounds in marine sediments. B.J. Robinson, M. Langille and J. Hellou. (PL) 62
Searching for StAR in an endocrine haystack: how is beta-sitosterol affecting cholesterol availability? R.L. Sharpe, J. Bonselaar, G.J. Van Der Kraak, A.J. Woodhouse, T.W. Moon and D.L. MacLatchy. (PL) 66
Effects of a potent estrogen on a freshwater food web. K.A. Kidd, D.L. Finley, M.J. Patterson, A.G. Salki, P.J. Blanchfield and K.H. Mills. (PL) 66
In vitro assessment of biological impacts to rainbow trout by the polycyclic musk HHCB. D.B.D. Simmons, C.D. Metcalfe, G.C. Balch, S. O'toole and J. Yang. (PL) 66
Endocrine disrupting effects in fathead minnows exposed to pharmaceuticals and municipal wastewater effluent. J.L. Parrott, B.R. Blunt, C.A. Sullivan and S.M. Rhodes. (PL) 67
Sewage: a perpetual source of pharmaceutical and personal care products in Atlantic coast estuaries. G. L. Brun, R. Losier, F.L. Comeau, H.B. Lee, C. Surette and P. Falleta. (PL) 67
Assessment of immune competency of rainbow trout exposed to municipal sewage effluent. B. Wasserab, M.R. van den Heuvel, B. Koller, B.C. Hitzfeld and D. Dietrich. (PL) 68
Effects of pharmaceutical products in aquatic organisms. F. Gagné and C. Blaise. (PL) 68
Aquatic toxicity of carbamazepine®, atorvastatin® and triclosan® to benthic invertebrates. È.B. Dussault, K.R. Solomon, E. Sverko and P.K. Sibley. (PL) 69
A re-assessment of wild fish from Canadian Areas of Concern for reproductive health. M.E. McMaster, G.R. Tetreault, C. Boyko, S.B. Brown and J.P. Sherry. (PO) 70
Measuring acidic and neutral pharmaceuticals in surface waters by GC-MS and HPLC-MS analysis. F.L. Comeau, G.L. Brun, C. Surrette and R. Losier. (PO) 70
Effects of testosterone and 5α-dihydrotestosterone on plasma IGF-1 and growth of Atlantic salmon smolts. J.T. Arsenault, W.L. Fairchild, D.L. MacLatchy, K. Haya, L.E. Burridge and S.B. Brown. (PO) 70

Toxicological evaluation of Georgia Basin municipal waste water effluents (MWWE). J. Bruno and G.C. Van Aggelen. (PO) 71
Targeted proteomics to measure vitellogenin in rainbow trout. M. Smith, M.R. van den Heuvel and N. Ling. (PO) 71

Artifactual Toxicity in Municipal Waste Water - Ammonia and pH / Toxicité artificielle des eaux usées municipales - ammoniaque et pH

Municipal experiences with apparent effluent ammonia toxicity. A. van Roodselaar, R. Ng, B. Hystad, G. Marsh and S. Bertold. (PL) 72
Criteria and supporting rationale for applying to use the pH stabilization procedure during the testing of acute lethality of municipal wastewater to rainbow trout. R.P. Scroggins, L.J. Novak and K.E. Holtze. (PL) 72
pH Control by carbon dioxide addition for Environment Canada's rainbow trout acute lethality test: a review and update of method development. G.C. van Aggelen and G.R. Schroeder. (PL) 72
Inter-laboratory toxicity evaluation of pH stabilization procedures for use with municipal wastewater. L.J. Novak. (PL) 73
Toxicological assessment of ammonia using Environment Canada's rainbow trout acute lethality test (RM9). G.R. Schroeder and G.C. van Aggelen. (PL) 73
Site-specific surface water quality objective for ammonia plus chloramine. G.R. Craig, I. Middelraad and D.G. Dixon. (PO) 74
pH Controller technology and it's application to pH stabilization in rainbow trout acute lethality tests. R. Chong-kit and J.E. Schroeder. (PO) 74
Changes in metal bioavailability and speciation along a municipal wastewater effluent dispersion plume. C. Gagnon, P. Turcotte and B. Vigneault. (PO) 74
Development of a methodology for testing the toxicity of ammonia to aquatic invertebrates at low temperature and low pH. D.G. Poirier, J. Van Geest and A. Tomczyk. (PO) 75

Sediment and soil toxicity/Tocoxité des sédiments et des sols

Validation of Environment Canada biological test methods for assessing contaminated soils: earthworm and plant toxicity tests. J.I. Princz and R.P. Scroggins. (PL) 75
Eisenia fetida or *E. andrei*: which species are you using? J.H. McCann and D.G. Dixon. (PL) 76
Use of an avoidance test with earthworms for screening reference soils and soils contaminated with petroleum hydrocarbons. N.C. Feisthauer, D.L. Holtze, J.T. Crumb and G.L. Stephenson. (PL) 76
Influence of ammonia, pH, dissolved organic carbon and other potentially confounding factors on sea urchin porewater toxicity tests. R.S. Carr, M. Nipper and J.M. Biedenbach. (PL) 77
Research needs to improve echinoid fertilisation and development bioassays in Environment Canada's *Disposal at Sea Regulations*. S. Agius. (PL) 77
Exposure of sphaerid clams to aquaculture waste in an *in situ* sediment bioassay. M. Kullman, K.A. Kidd and C. Podenski. (PL) 77

Evaluating a suite of sediment toxicity tests to aid in the development of sediment quality guidelines for use by the Disposal at Sea program. D. Lee, K.G. Doe, G.C. van Aggelen, C. Buday, L. Meloche and C. Wong. (PO) 78
Assessing the toxicity of refrigerated and frozen stream sediments using Japanese medaka embryo-larval bioassays. C.F. Jardine and K.L. Teather. (PO) 79
Evaluating the ecological relevance of sediment quality guidelines. C. Wong. (PO) 79

Small bodied fishes - application to environmental monitoring / Les poissons de petite taille - utilisation dans les suivis environnementaux

Toxicity and EROD-inducing potency of alkylated polycyclic aromatic hydrocarbons (PAHs) in fish. D. Turcotte, M. Bowerman, P.V. Hodson and R.S. Brown. (PL) 79
Measuring multixenobiotic resistance *in vivo* in fish. S.M. Bard and J.J. Stegeman. (PL) 80
A search for the cause of reproductive steroid depressions in fish exposed to pulp mill effluents. K.S. Shaughnessy, A.M. Belknap, L.M. Hewitt and D.L. MacLatchy. (PL) 80
Use of stable isotopes to examine the site fidelity of mummichogs (*Fundulus heteroclitus*) in an Atlantic Canadian estuary receiving multiple anthropogenic influences. M.A. Skinner, S.C. Courtenay, W.R. Parker, M.G. Dubé and R.A. Curry. (PL) 81
Assessing effects on fish from a stream receiving oil refinery effluent. G. Vallieres, D.L. MacLatchy and K.R. Munkittrick. (PL) 81
Biology of multi-spawning, small-bodied fish species with sampling considerations for environmental monitoring programs. B.J. Galloway and K.R. Munkittrick. (PL) 82
Using small-bodied fish in effects-based assessments: interpreting non-lethal data for use in environmental monitoring studies. S. Brasfield, M.A. Gray and K.R. Munkittrick. (PL) 82
Complex study designs utilizing small, short-lived species reveal complex patterns: are we gaining insight, or just muddying the waters? C.B. Portt, B.W. Kilgour, G. Rogozinski and C. Turpin. (PL) 83
Using environmental gradients to predict reference conditions: a case study with Toronto-area stream fish communities. B.W. Kilgour and L. Stanfield. (PL) 83
Examining population-level responses in small-bodied and short-lived fishes: what *Cottus* spp. has taught us. S. Brasfield, K.R. Munkittrick and C.B. Portt. (PO) 84

Contaminants in aquatic systems / Les contaminants présents dans les systèmes aquatiques

Selenium from coal mining in the Elk River Valley, British Columbia, Canada. P.M. Chapman. (PL) 84
Investigation of selenium uptake pathways in lentic and lotic aquatic habitats using stable isotope analysis. P.L. Orr, K. Guiguer and C.K. Russel. (PL) 85
Temporal trends in mercury and organic contaminants in coho and chinook salmon from Lake Ontario. L.M. Campbell, T. French, D. Jackson, W. Schneider and 85

A. Hayton. (PL)
Control of mercury emissions from chlor-alkali plants in India. S. Pandey. (PL) 85
Creososte contamination in the Grey Owl Marina, Prince Albert National Park: PAH and benthos distributions. M.S. Evans and K. Fazakas. (PL) 86
Non-destructive sampling for chemical analysis of fish tissues. K. Connors and C.K. Russel. (PL) 86
Assessment of cumulative impacts in three species of fish in a New Zealand river. D.W. West, M.R. van den Heuvel, N. Ling, B. Hicks and L. Tremblay. (PL) 87
Zooplankton community structure and aquatic chemistry in northeastern Alberta: potential sensitivities to increased acidification. M.S. Evans, D. Andrews, D. Jeffries and P. McEachern. (PL) 87
Chlorine-based disinfection of primary municipal wastewater - a review of practices, efficacy, monitoring and environmental impact. C.S. Bottaro and K.A. Hawboldt. (PL) 88
Food web biomagnification of brominated diphenyl ethers in two Great Lakes fish communities. D.M. Whittle, D.C. MacEachen , D.B. Sergeant and M.J. Keir. (PO) 88
Species differences in the bioaccumulation relative to biotransformation of polycyclic aromatic hydrocarbons. J. Hellou, K. Campbell, K. Cheeseman, A. Gronlund, R.D. Guy, J. Leonard, L. Ramaley and S. Steller. (PO) 89
Spatial and seasonal dynamics of polychlorinated biphenyl (PCBs) transport and bioaccumulation using freshwater mussels. G.R. Craig, I. Middelraad, M.H. Salazar, S.M. Salazar and C.B. Portt. (PO) 92
Nine-year review of Gulfwatch in the Gulf of Maine: trends in tissue contaminant levels in the blue mussel, *Mytilus edulis*, 1993–2001. L. White, P.G. Wells, S. Jones, C. Krahforst, G.C. Harding, P. Hennigar, G.L. Brun and N. Landry. (PO) 93
Polychlorinated biphenyls levels in sport fish from Lake Erie. A. Kielaszek, T. Franks, B. Schepart and P.F. Dehn. (PO) 93
Mercury in sport fish from Lake Erie. R. Schuster, D. Weibel, K. Burke, L. Shepherd, G. Smietana, B. Schepart and P.F. Dehn. (PO) 94
Use of small fish in caged field experiments. V.P. Palace. (PO) 94

Biological effects / effet biologique

Glutathione *S*-transferases as a pathway for protecting against oxidative stress in fish. E.P. Gallagher. (PL) 94
Temperature influences polycyclic aromatic hydrocarbon (PAH) solubility, pharmacokinetics, and CYP1A activity in fish. J.A. Dungavell and P.V. Hodson. (PL) 95
Chronic aquatic toxicity of alcohol ethoxylate surfactants under Canadian exposure conditions. S.E. Belanger and P.B. Dorn. (PL) 95
Occurrence and persistence of Cry1Ab genes in the aquatic environment. F. Gagné, C. André and C. Blaise. (PL) 104
Expanding the application of field-based mesocosms for life cycle studies with benthic invertebrates and partial life cycle studies with fish. M.G. Dubé, C.J. Rickwood, K.A. Hruska, D.L. MacLatchy and N. Glozier. (PL) 105
Is the Burrard inlet homogenous? What the mussels are telling us. S.D. St-Jean, F. Bishay and P. van Poppelen. (PL) 106

Importance of dietary uptake on metal toxicity to *Hyalella azteca*. A.L. Wallace, U. Borgmann and D.G. Dixon. (PL) 106
Further characterization of a metal-binding, histidine-rich glycoprotein (HRG) from the blood of marine bivalve molluscs. W. Robinson, M. Sugumaran, G. Wallace, A. Abebe and S. Catanzano. (PL) 106
Influence of hardness on aqueous uranium toxicity. K.C. Serben, C.V. Eickhoff, G.A. Bird and S. Munger. (PO) 106
Are fish in areas of concern (AOCs) on the Canadian side of the Great Lakes exposed to estrogens? J.P. Sherry, C. Tinson, M.E. McMaster and S.B. Brown. (PO) 107
Microcystin-LR effects on the cytoskeleton of the human hepatoma (HepG2) cell line. J. Cercone, E. Welhhofer and P.F. Dehn. (PO) 107
Wood leachate concentration in waters from the Pacific coast, are there effects on the health of mussels, *Mytilus trossulus*. S.D. St-Jean, M. Kohli and P. van Poppelen. (PO) 108
Non-professional phagocytosis in fish response to pathogens: roles of stress and toxicant exposure. L.E.J. Lee, R. Donkersgoed and R.M. Slawson. (PO) 108

Environmental Effects Monitoring - metal mining / Étude de suivi des effets sur l'environnement - mines de métaux

Effects of water chemistry on copper toxicity to *Mytilus* sp. - implications for saltwater quality criteria in the USA. W.R. Arnold. (PL) 108
Modelling metal-gill interactions and metal toxicity to fish: the influence of natural organic matter source. R.C. Playle. (PL) 109
Environmental study at a closed mine where regulated effluent pH limits exceeded natural pH levels. C.K. Russel, P.I. LePage and P.L. Orr. (PL) 109
Contamination of freshwater and marine environments from historical gold mining activities in Nova Scotia. M.B. Parsons, P.K. Smith, T.A. Goodwin, G.E.M. Hall, S. Witch, V.P. Palace, R. Parrott and A.L. Sangster. (PL) 109
Use of small forage fish in caged exposures for metal mining Environmental Effects Monitoring (EEM). C. Doebel, C.L. Baron, K.G. Wautier and V.P. Palace. (PL) 110
Harmonization of provincial and federal requirements for environmental effects monitoring at the Eskay Creek Mine, British Columbia. B.G. Wernick, P.M. Chapman and R. Martel. (PL) 111
Development and implementation of a *Metal Mining Effluent Regulations* - Environmental Effects Monitoring (EEM) program at a uranium mine in northern Saskatchewan. M.A. Balych and K.D. England. (PL) 116
Setting ecological and risk-based corporate performance targets for developing tailing-management plans. J.M. McKernan and D.F. Stewart. (PL) 116
Summary of 2003 metal mining effluent characterization, toxicity, and water quality monitoring data in Pacific and Northern Region. S. Blenkinsopp, J-M. Ferone, P. Siwik and G. Groskopf. (PO) 117
The Greater Northern Ontario Mining Effects Study - reference site analysis. M.F. Bowman, C. Brereton, W. Keller and K.M. Somers. (PO) 117
Influence of natural organic matter quality on aluminum binding to gills of 121

rainbow trout. A.R. Winter, T.A.E. Fish and R.C. Playle. (PO)
The effects of natural organic matter source on silver uptake by gills of rainbow trout. T.A.E. Fish, A.R. Winter and R.C. Playle. (PO) 122
The bioaccumulation of select metals and metalloids in a smelter impacted aquatic ecosystem. L. Desbiens, G.A. Spiers and J. Gunn. (PO) 122
Use of surface water, pore water and sediment bioassays with *Hyalella azteca* to identify the cause of benthic impacts at a Saskatchewan uranium mine/mill. E.L. Robertson and K. Liber. (PO) 122

Serendipity in aquatic sciences / Les découvertes fortuites en sciences aquatiques

Serendipity is the future of aquatic ecotoxicology. P.M. Chapman. (PL) 123
Aquatic toxicity of petroleum: an evolving paradigm. J.M. Neff. (PL) 123
Marine natural products, a valuable source of chemical diversity for drug discovery research (sometimes it is better to be lucky than it is to be good). K.R. Gustafson. (PL) 124
Potential catastrophic legacy: the case of the missing chlorine tankers. S.M. Bard and A. Edwards. (PL) 124
The role of serendipity in interdisciplinary environmental research. M.B. Parsons. (PL) 125
Maximizing serendipitous scientific discoveries. K. Hedley. (PL) 125
Serendipity and environmental science: is there a place for serendipity in science? J. Hellou, P.M. Chapman, J.M. Neff, K.R. Gustafson, S. Bard, M.B. Parsons and K. Hedley. 125

Best student paper awards / Prix les meilleurs exposés par des étudiants 128

List of participants / Liste des participants 129

Workshop Proceedings / Compe rendus d'atelier 137

Plenary / Plénière

Session co-chairs: L.E. Burridge and K. Haya

A vision for aquatic toxicology in the Department of Fisheries and Oceans. S. Paradis. Fisheries, Environmental and Biodiversity Science Directorate, Department of Fisheries and Oceans, Ottawa, ON.

Agricultural practices in Prince Edward Island for a sustainable environment. R.M. Cheverie. Prince Edward Island Department of Agriculture, Agriculture Resource Division, Charlottetown, PE.

A number of fish kills linked to agricultural run off has initiated a change in how farmers produce row crops in Prince Edward Island. Buffer zone legislation, crop rotation legislation and changes to the pesticides control act were designed to protect surface and groundwater in the province. The Sustainable Resource Conservation Program offers matching funds to producers to address potential environmental hazards on their farm, identified in their Environmental Farm Plans as well as incentives for following integrated pest management and nutrient management programs. Over 1300 projects have been funded to date.

Water on Prince Edward Island: precious resource or abused commodity? D.L. Guignion. Department of Biology, University of Prince Edward Island, Charlottetown, PE.

Prince Edward Island (PEI) consists of flat to rolling farm land with approximately one-half of the province interspersed with successional forests. The 1800 km of coastline is heavily indented with bays and estuaries. Formed from eroded mountains and deposition of sediments in the southern Gulf of St. Lawrence, the Island is underlain with fractured sandstone bedrock. Enormous quantities of fresh water are present in the aquifers and total recharge on an annual basis is normal. PEI has over 200 short spring-fed streams, 80 of which are greater than 3 m in width where they flow into estuaries. Salmonids predominate the fish fauna in fresh water while the estuaries serve as nurseries for over two dozen species of fish, many of commercial importance. A thriving shellfish industry relies on high quality estuarine water.

In the 1970s, a "development plan" encouraged agriculture to move towards monoculture of potatoes. The construction of two processing plants in the 1980s also changed farming dramatically. Larger fields, shorter crop rotations, lower organic matter and fewer farmers became the norm. Soil erosion, sedimentation of watercourses, and elevated nutrient levels are major environmental problems that plague the Island in spite of environmental farm plans and increasing awareness of the issues. New legislation, including a three year crop rotation and riparian buffer zones, are porous and most fresh water fish kills occur during intense rainfall events where all of the best management practices would need to be in place to contain run-off. Nutrient management is an even greater challenge than soil erosion. In 2003, levels of nitrate-nitrogen in four drainage basins which are mostly forested averaged about 0.2 mg/L; in the Wilmot River, situated in the potato belt, nitrate levels averaged 7.3 mg/L. There is a strong correlation between the percentage of drainage basins in potato production and nitrate levels in streams. Over-enrichment in estuaries and anoxic events are increasing, with 26 documented occurrences in 2003.

In a province totally reliant on groundwater, water is truly a most precious resource. Threats to ground water and surface water posed by point and non-point source pollution, regional over-extraction, high rates of sedimentation and over-enrichment must be dealt with expediently before impacts further degrade our resources and way of life.

National Agri-Environmental Standards Initiative for the Agricultural Policy Framework. J.L. Papineau[1] and J. Jarjour [2]. [1]Environment Canada, Water Policy and Coordination Directorate, Gatineau, QC; and [2]Environment Canada, Conservation Priorities Directorate. Gatineau, QC.

In June 2002, Agriculture Ministers from federal, provincial and territorial governments agreed to accelerate action towards common environmental goals for biodiversity, air quality, pesticides use, soil quality and water quality & conservation. Through the Agricultural Policy Framework (APF), the goal is to brand Canada as the world leader in environmentally responsible production, in food safety and quality, and innovation. The APF is composed of 5 chapters: Business and Risk Management, Renewal, Food safety and quality, Science and Innovation and, Environment.

The National Agri-Environmental Standards Initiative (NAESI) is a 4 year program under the Environment chapter. Environment Canada (EC) has been given the mandate to develop and deliver agri-environmental performance standards that are consistent with broader national goals, policy, legislation and other environment programs.

The NAESI has the following goals: (1) to support a consistent national approach to the achievement of common environmental outcomes over the long term, (2) to support the move towards environmental farm planning and farm certification; and (3) enhance the environmental performance and stewardship of the agriculture sector.

The standards will be practical and consistent science-based benchmarks to help guide the design of farm practices in achieving desired environmental outcomes. They are not intended as regulatory instruments. Two types of Environmental Performance Standards (EPS) will be developed by EC: ideal standard and achievable standards. AAFC will provide the process standards; also know as Beneficial Management Practices (BMPs).

NAESI consists of a two phase approach. The Scoping year (2004-2005) includes an extensive scoping exercise with key priorities: (1) identify and assess key agriculture pressures and high risk sectors to determine priorities; (2) assess and evaluate existing standards/guidelines, certification systems etc, (international, national and provincial levels); (3) review and/or design methodologies for standards development; (4) demonstrate early action in key areas through pilot projects (for example: programs such as Watershed Evaluation of BMPs); and (5) integration with the APF-Environment chapter programs. Phase two (2005-2008) encompasses the development of Performance Standards which includes their testing, assessment, validation and implementation.

Each of the four themes (air, biodiversity, water and pesticides) has their specific environmental quality objectives/goals. The Air theme will develop Ideal/Achievable Performance Standards that reduce farm contribution to airborne particulate matter, greenhouse gases and odour. The Biodiversity theme will focus on the quality and quantity of habitat that actually produces aquatic and terrestrial biodiversity and, aim to reduce the negative effects of agricultural practices on nature, and of nature on agriculture. The Pesticides theme's objectives are to reduce the risk of pesticide impacts through the use of reduced risk pesticides and, change the behaviour from a reliance on pesticides to Integrated Pest Management. The Water theme will develop EPS for pathogens, nutrients, water conservation, instream flow needs and sediment infux.

Desired outcomes for the NAESI include: (1) achievement of environment quality objectives via the development of EPS; (2) providing tools and techniques to relate Performance Standards to Process Standards; (3) delivering standards that can assist farmers in enhancing their environmental performance and to report environmental performance against; integration of EC's priorities within the suite of APF-Environment Chapter programs; and (4) helping the farming community move towards achieving sustainable agriculture and environmental farm certification.

The Agricultural Policy Framework will provide benefits to the agriculture sector by providing it with a reputation for environmental responsibility, green marketing opportunities and improvement to the bottom line (for example: more productive soil and lower environmental liability). The Economy will benefit with the move towards a more vibrant and sustainable agricultural industry and, reduced environmental costs transferred from agriculture to other economic sectors. Lastly, Canadians will benefit from a healthier environment, a higher quality of life and, from sustainable management of natural resources for future generations.

Contributed Papers/Documents contribués

Environmental effects monitoring (EEM) - pulp and paper / Étude du suivi des effets sur l'environnement - pâtes et papiers

Session co-chairs: S.C. Courtenay and M.H. Murdoch.

Overview of Cycle 3 findings of aquatic effects due to pulp and paper mill effluents. B.L Ring[1], K. Hedley[1], R.B. Lowell[2], G. Pastershank[1], L. Trudel[1] and S.L. Walker[1]. [1]Environment Canada, National Water Research Institute, Gatineau, QC; and [2]Environment Canada, National Water Research Institute, Saskatoon, SK.

Canadian Pulp and Paper mills are required under the *Pulp and Paper Effluent Regulations* to conduct Environmental Effects Monitoring (EEM) studies on their receiving environment. Fish and benthic invertebrate studies are conducted to assess the potential effects of effluent on fish, fish habitat, and the use of fisheries resources and sublethal toxicity data is used to assess overall changes in effluent toxicity. The National Environmental Effects Monitoring Office is currently completing analyses of the Cycle 3 studies. This presentation will provide an overview of Cycle 3 EEM submissions: describing exemptions, study designs and alternatives used, common errors in data submission, and a summary of the sublethal toxicity results and the type and magnitude of effects on key fish and benthic invertebrate endpoints. After extensive analysis of Cycle 2 EEM biological data, Environment Canada developed critical effect size guidelines for key fish and benthic invertebrate endpoints. The relevance of these effect sizes for Cycle 3 studies will be presented, including the number of mills exceeding them.

Pulp and paper mill effluent effects on invertebrates: national response patterns over two Environmental Effects Monitoring cycles. R.B. Lowell[1], B.L. Ring[2], G. Pastershank[2], L. Trudel[2] and K. Hedley[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; and [2]Environment Canada, National Water Research Institute, Gatineau, QC.

The National Environmental Effects Monitoring (EEM) Program has just completed Cycle 3 of pulp and paper mill effluent monitoring. Meta-analyses were used to evaluate the magnitude and patterns of effects on benthic invertebrate communities in Cycle 3, and these findings were compared to those from Cycle 2 of the EEM Program. In Cycle 2, the most common response pattern observed in benthic invertebrate communities was one of mild to moderate eutrophication, although more pronounced eutrophication, as well as toxic or smothering effects, were observed in some areas. The eutrophication was likely due to the nutrient enrichment effects of the phosphorus, nitrogen, and organic content of the effluent. The overall response patterns were similar in Cycle 3, with some endpoint/habitat combinations showing almost identical responses to those observed in Cycle 2. There were also, however, indications of changes in the degree of response in some habitat types. These included evidence of both increased effects (freshwater depositional habitats) and recovery from effects (marine habitats) between Cycles. These data provide a foundation for future investigations of long-term trends in the nature of pulp and paper mill effluent effects on receiving water biota.

National assessment of pulp and paper mill effluents on fish: comparison between two Environmental Effects Monitoring cycles. L. Trudel[1], G. Pastershank[1], R.B. Lowell[2], B.L. Ring[1] and K. Hedley[1]. [1]Environment Canada, National Water Research Institute, Gatineau, QC; and [2]Environment Canada, National Water Research Institute, Saskatoon, SK.

The second round (Cycle 2) of Environmental Effects Monitoring studies (EEM) were completed by pulp and paper mills between 1996-2000 and the findings were summarized in a national assessment report. In Cycle 2, the national average response pattern observed in fish consisted of a decrease in gonad weight and increases in liver weight, condition factor, and weight-at-age. This was determined by conducting meta- and multivariate analyses on key endpoints for 56 EEM fish survey studies: up to two species of fish per mill, males and females, collected at reference and exposure sites. These response patterns were consistent with some degree of metabolic disruption coupled with nutrient enrichment. Cycle 3 EEM fish survey studies were completed by mills between 2000-2004. Similar statistical analyses as used for Cycle 2 are being applied to the 58 fish studies completed in Cycle 3, and a comparison between the results of the two Cycles will be presented. A comparison of the fish survey data permits us to determine whether or not the same types and magnitudes of effects exist between the two Cycles.

Fish'n with variability in the Athabasca River. R. Shelast[1] M. Luoma[1] and M. Spafford[2]. [1]Stantec Consulting Ltd., Calgary, AB; and [2]Alberta-Pacific Forest Industries, Boyle, AB.

Natural variability has been a dominating factor during all adult fish surveys for the Alberta-Pacific Forest Industries pulp mills Environmental Effects Monitoring program (EEM) on the Athabasca River. Depending on the sample size and age of the catch results can vary from cycle to cycle. Use of alternative methods (e.g., non-lethal sampling) of fisheries assessment have also been attempted and show similar flaws. Benthic invertebrate surveys including the assessment of periphytic algae and water quality conducted during these same EEM cycles have robust sample sizes, are easily reproduced and have more sensitivity to water quality changes than fisheries surveys. In this case the use of benthic surveys to predict impacts on fisheries makes sense and benthic invertebrates appear to talk louder than fish for EEM.

Patterns of benthic diversity and nutrient enrichment in a tidal river receiving environment for a pulp and paper mill in New Brunswick. M.H. Murdoch[1], M. Stephenson[1], A. Gallant[2] and J-Y.Ritchie[3]. [1]Jacques Whitford Environment Ltd., Fredericton, NB; [2]AV Cell Dissolving Sulphite Mill, Atholville, NB; and [3]Tembec Inc., Temiscaming, QC.

Cycle 3 Environmental Effects Monitoring (EEM) studies conducted at the AV Cell Dissolving Grade Sulphite Mill in Atholville, NB, revealed a pattern of increasing benthic diversity associated with nutrient enrichment in the sediment. The mill discharges secondary treated effluent via a channel into an estuarine environment on the south shore of the Restigouche River. The benthic invertebrate community survey used a "gradient to background" design. Sampling locations included four upstream and four downstream stations along a near shore exposure gradient, and four shallow reference stations on the north side of the estuary, matched as closely as possible to the exposure stations in sediment grain size and other habitat characteristics. Benthic invertebrate diversity was significantly higher in the exposure area than in the reference areas, and the diversity response was greatest at stations 100 m upstream and 400 m downstream of the effluent discharge to the Restigouche River. This response is considered an effect of nutrient enrichment to the effluent discharge. There was little or only weak evidence for effects of the mill effluent on benthic inverterate abundance and richness.

Comparing apples to orchards: taxonomic resolution and Environmental Effects Monitoring (EEM) marine benthos effects assessment. L. Uhlig, M. Davies, M. Ptashynski and W.N. Gibbons. Hatfield Consultants Ltd., West Vancouver, BC.

The Updated Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring (EEM) recommends that family level identification be used for freshwater benthic invertebrate surveys for the detection of effects from effluent (Environment Canada 2004). However, for marine/estuarine environments, the document recommends that all benthic invertebrates be identified to the lowest practicable level (LPL), and states that "more study and specific attention to the effects of mills on benthic biodiversity" are needed before changing to higher taxa levels. We conducted analyses on three datasets from pulp and paper EEM programs at marine mills to determine what would be the results if invertebrates were reported at more generalized levels than LPL, specifically family level. Results included: the taxa list was reduced by 43-52%; evenness and Bray-Curtis index values were lower in general; and regression analyses resulted in similar statistical outcomes regarding effects, with similar *p* values. Costs and effort would be reduced by 25 to 30% for family level identification relative to LPL for marine benthic programs, thereby making them more similar in size and cost to freshwater benthic programs.

Methods

Cycle 3 benthic invertebrate data from three mills in southwest BC were used for this analysis. All three mills used gradient designs with 11-15 stations per study and 2-4 replicates per station. Invertebrates were enumerated at the lowest practicable level (LPL) by taxonomists. For each mill, taxa were "rolled up" into the family level and endpoints (richness, evenness and Bray-Curtis indices) were recalculated. Densities remained the same for each station. Regression analyses were conducted on family level endpoints; data were transformed if necessary to meet test assumptions. Family level *p*-values and effects determination were compared to LPL results.

Results

In general, family level taxonomic resolution relative to LPL resulted in reduced richness (43-52% fewer taxa per survey), lower evenness indices in general (but some variability was observed), and lower Bray-Curtis index values for each station.

Table 1. Powell River Cycle 3 benthic endpoints: p-values and effects.

	LPL	Effect	Family	Effect?
Density	0.65	No	0.65	No
Richness	0.55	No	0.65	No
Evenness	0.26	No	**0.056**	**Yes**
Brayt-Curtis	0.14	No	0.29	No

p-value <0.1 considered significant

Powell River Cycle 3 data reported 314 taxa at LPL and 136 taxa at the family level. No significant effects were observed along a distance gradient using LPL resolution (Hatfield Consultants Ltd. 2004a). At family level, evenness became significant (p=0.1) (Table 1).

Port Alberni Cycle 3 data reported 205 taxa at LPL and 98 taxa at family level. With LPL, significant effects were observed for richness and the Bray-Curtis index along a distance gradient (Hatfield Consultants Ltd. 2004b); these same effects were observed with family level taxonomic resolution (Table 2).

Table 2. Port Alberni Cycle 3 benthic endpoints: p-values and effects.

	LPL	Effect?	Family	Effect?
Density	0.24	No	0.24	No
Richness	**<0.001**	**Yes**	**<0.001**	**Yes**
Evenness	0.77	No	0.59 ($\log_{10}$)	No
Bray-Curtis	**<0.001**	**Yes**	**0.006**($\log_{10}$)	**Yes**

p-value <0.1 considered significant

Crofton Cycle 3 data reported 293 LPL taxa and 153 family level taxa. A significant effect for density only was observed with LPL data along a pulpmill gradient (C:N ratio) (Hatfield Consultants Ltd. 2004c). This did not change with family level resolution. The other three endpoints indicated no effect at both levels of taxonomic resolution (Table 3).

Table 3. Crofton Cycle 3 benthic endpoints: p-values and effects.

	LPL	Effect?	Family	Effect
Density	**0.084**	**Yes**	**0.08**	**Yes**
Richness	0.79 (rank)	No	0.58	No
Evenness	0.82 ($\log_{10}$)	No	0.42	No
Bray-Curtis	0.14 ($\log_{10}$)	No	0.15 ($\log_{10}$)	No

p-value <0.1 considered significant

In summary, effects were consistent between the two taxonomic levels except for evenness at Powell River. Evenness was more variable, with some indices higher at family level relative to LPL. There was no clear pattern between the reduction in number of taxa per station and whether evenness was higher or lower at the family level.

Costs and effort to sort, identify and analyze marine benthic invertebrate samples have been considerably higher relative to freshwater benthic invertebrate programs. Given marine surveys report approximately 300 taxa per mill, using family level taxonomic resolution would result in a 25-30% reduction in costs relative to LPL. Accordingly, less laboratory time would be required, and data would be available sooner (e.g., within several months rather than one year). In comparison, freshwater benthic invertebrate surveys on the west coast reported between 80-100 taxa at LPL. Costs for LPL analyses of freshwater samples are approximately 1/3 of marine samples to LPL. Therefore, by using family level for marine surveys, the marine and freshwater invertebrate surveys become more similar in numbers of taxa and overall costs.

Conclusions

Marine invertebrate surveys can benefit by using family level identification without compromising effects determination. No "rare" taxa are lost as counts are included at the family level. Therefore, any noise that may occur in analyses due to the high number of rare taxa is reduced by using family level data. Costs and laboratory efforts also are reduced when family level taxonomic resolution is employed.

Given the constant revision of species names and groupings, it is difficult to maintain accurate lists of taxa over time and space. Family level identification would likely provide a more consistent database of taxa that could be used nationally and historically for the EEM program.

Acknowledgements

Cycle 3 programs were funded by Powell River, Port Alberni and Crofton Divisions of NorskeCanada. Taxonomists included Biologica Environmental Services and Columbia Science.

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Investigation of cause in pulp and paper Environmental Effects Monitoring. L.M. Hewitt[1], M.G. Dubé[2], S.C. Ribey[3], J.M. Culp[4], B.W. Kilgour[5], C.B. Portt[6], K. Hedley[3], D.L. MacLatchy[7] and K.R. Munkittrick[7]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Environment Canada, National Water Research Institute, Gatineau, QC; [4]Environment Canada, National Water Research Institute, Fredericton, NB; [5]Jacques Whitford Environment Ltd., Ottawa, ON; [6]C. Portt and Associates, Guelph, ON; and [7]Department of Biology, University of New Brunswick, St. John, NB.

Environmental Effects Monitoring (EEM) has completed three cycles in the pulp and paper sector and is initiating its first cycle for metal mining. We have composed a guidance document that contains a tiered framework for investigating cause where responses are persistent or getting worse. A causal investigation will have different outcomes, depending on the type of effect, its severity, social factors, economic factors and technical factors. A determination of cause ultimately ends with the confirmation of individual chemicals present in a effluent that elicit the responses observed in conducting EEM studies. It is our contention that this level of detail is not required at all sites where differences have been noted, nor is it economically feasible. We propose that the depth to which an investigation of cause is conducted be determined on a site-specific basis which is decided upon by stakeholders. Our framework consists of assessment tiers in courses of action based on either eutrophication responses or contaminant based responses. The objective of nutrient-based investigations is to identify the nutrient form and then its source within the mill. Contaminant responses are more detailed and involve investigating individual process effluents within the industrial facility to determine their relative contributions to the effects, with additional steps to isolate and identify the individual causative chemicals. This framework within the guidance document can be used in a stakeholder decision-making process to determine the extent of the investigation. Intensive research projects utilizing these approaches at selected sites are providing a database of causative processes and/or substances that will be useful in conducting future investigations.

Use of fathead minnow (*Pimephales promelas*) in assessing reproductive effects of pulp mill effluent for investigation of cause studies. C.J. Rickwood[1], M.G. Dubé[2], D.L. MacLatchy[3], L.M. Hewitt[4] and J.L. Parrott[4]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Department of Biology, University of New Brunswick, St. John, NB; and [4]Environment Canada, National Water Research Institute, Burlington, ON.

Treatment of final pulp mill effluent (PME) has made significant improvements in effluent toxicity, however, reproductive responses in wild fish continue to be documented. There is a pressing need to identify the source or cause of these responses so process/treatment changes or other mitigation options can be explored. The objectives of this study were to: (1) identify consistent sensitive endpoint(s) in a modified fathead minnow (FHM) bioassay that could be used in investigation of cause studies with final PME, and (2) determine the process waste stream(s) that was the possible principle source of compound/s causing reproductive effects observed after exposure to final PME. Reproductive output of FHM breeding pairs was assessed throughout a 21 day pre-exposure and 21 day exposure period. Treatments included final PME (100% and 1%) and four selected process streams, at various concentrations, selected using a flow proportion approach based on acute toxicity studies. Consistent, sensitive endpoints were selected to identify the possible process stream that was the source of compound/s causing reproductive effects observed in FHM after final PME exposure. Obtaining pre-exposure data and the use of pair-breeding FHM in this assay also gave a sensitive indication of effluent effects and allowed accurate endpoint comparisons to be made.

Statistical analysis tool (SAT) for Environmental Effects Monitoring analysis – how cool is this? S.C. Ribey[1], M.G. Dubé[2], R.B. Lowell[2], G.R. Champagne[1], I. Wong[3] and J. Inkster[2]. [1]Environment Canada, National Water Research Institute, Gatineau, QC; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

The National Water Research Institute has developed a statistical software package that can produce and synthesize the key Environmental Effects Monitoring (EEM) endpoints in a consistent manner. A generic model of this statistical analysis tool (SAT) was initially developed using data from Cycle 2 pulp and paper EEM, with the five mill northern Alberta study. This early model used data that needed processed and extensive QA/QC from the Excel spreadsheet EEM electronic data submission. The SAT has evolved and is now capable of retrieving data mill by mill from the EEM data entry software. SAT can conduct ANOVA and ANCOVA analyses for control impact study designs for fish and benthic invertebrates. As with other statistical software packages, SAT tests for residuals, allows for the removal of outliers, allows data transformation, generates graphics for the comparisons, and produces site effect summary tables. The SAT has gone through vigorous testing since the submission of Cycle 3 pulp and paper data. The SAT will aid in standardized and reliable data assessment, which will allow for more timely national and regional analysis of EEM data. This presentation will include a demonstration of the SAT for EEM.

Pulp and paper mill effluent nutrient contributions to receiving waters in relation to biological effects at a watershed scale. T. Hall and W. Arthurs. National Council for Air and Stream Improvement (NCASI), Anacortes, WA.

Effluent nutrient contributions to two coldwater (McKenzie and Willamette Rivers, OR) and two warmwater (Leaf River, MS and Codorus Creek, PA) streams in the U.S. were assessed with respect to background nutrient concentrations over stream gradients that included tributary streams, point source pulp and paper mill discharges, as well as non-point nutrient sources. Based on measurements of total nitrogen (TN) and total phosphorus (TP) and the resulting ratio of N:P, three of the streams (McKenzie, Willamette, and Leaf) were indicated to be N limited (N:P < 6:1) and one stream (Codorus) to be highly phosphorus limited (N:P > 40:1). An assessment of seasonal samples collected over a 5-6 year period of time indicated a poor correlation between ambient or effluent standing crop measured as chlorophyll *a*. The only significant correlation was for the P limited stream where a significant correlation was indicated not for TP but for TN. These data were also used to test the validity of ecoregional nutrient criteria being proposed by the U.S. EPA in addressing nutrient concerns and that are based on two causal (TN and TP) and two response (chlorophyll *a* and turbidity) variables. The experience with these four receiving waters argues against the application of simple nutrient criteria or simple assumptions about nutrient related effluent effects.

A decade later: intertidal diversity is increased as pulp mill pollution is decreased.
S.M. Bard, F. De Raedemaecker and W. Willems. Faculty of Science, Dalhousie University, Halifax, NS.

Intertidal species diversity was evaluated along a pulp mill pollution gradient in British Columbia, Canada in 2004 in three regions: Howe Sound, Powell River and Prince Rupert. Species richness data was compared to a baseline study conducted in 1990-1993 using the same methodology and quadrat sites (Bard 1998). In all three regions, intertidal species richness decreases significantly as distance to a pulp mill decreases. In each region, mean species richness has increased over the past decade. These temporal increases in biodiversity are coincident with decreases in biochemical oxygen demand, total suspended solids, and adsorbable organic halide levels in pulp mill effluent. Intertidal diversity monitoring is a sensitive tool for assessing the impact of mill effluent on the marine environment. Depleted sites impacted by historical contamination that are in need of remediation can also be identified. The lowest diversity sites were negatively impacted by physical disturbance from accumulated wood debris. We advocate the adoption of intertidal monitoring for Pacific region pulp mills with marine outfalls as a complement to the current environmental effects monitoring (EEM) program initiated by Environment Canada. We recommend that in addition to monitoring current effluent discharge, that the EEM mandate include concrete steps for remediation of historical contamination by the pulp and paper industry and suppliers.

Proteomic profiling of teleost cell lines for high throughput aquatic toxicity assessment. S.K. Wagg and L.E.J. Lee. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

The protein profiles of several teleost species were compared in an effort to evaluate species and tissue specific responses to chemicals. Abietic acid (AA) is a wood derived chemical commonly found in pulp and paper mill effluents. AA is also a model carboxylic acid that has been tested as surrogate naphthenic acid. Naphthenic acids (Nas) are the main chemicals found in oil sands tailings that has been shown to be noxious to biota at cellular and organismal levels (see papers by Nero *et al.* and Dayeh *et al.* in this Proceedings). AA has been shown to be toxic to a variety of aquatic organisms and its acute mode of action has been attributed to its surfactant properties thus affecting proper functioning of cells. Comparison of cellular proteomes using cell lines has facilitated evaluation of toxicant action because of their ease of handling and manipulation. The effects of AA at the cellular level have not been studied in detail and this study aims to research the effects of AA at the fish cell proteome level. Several common proteins were observed to change but also some tissue specific proteins, as observed from 2D electrophoresis were noted to change. Towards this goal, several teleost fish cell lines were evaluated for visible differences in their of 2D gels from control and AA treated samples could be used as rapid signature profiling of toxicity.

Nutrient - algal accrual dose response curves using pulp mill and municipal effluents: an investigation of cause scenario on the Wapiti River, Alberta, Canada. N.E. Glozier[1], J.M. Culp[2], K.J. Cash[1], B.K. Firth[3] and G. Wilson[4]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Fredericton, NB; [3]Weyerhaeuser, Tacoma, WA; and [4]Weyerhaeuser, Grande Prairie, AB.

In the national assessment of pulp and paper Environmental Effect Monitoring studies, the most common national response pattern observed for benthic invertebrate communities was one of mild to moderate eutrophication likely due to nutrient enrichment. The Wapiti River, Alberta, an ultra-oligotrophic system, provided an excellent opportunity in which to investigate the relationship between effluent nutrient levels and algal biomass accrual in sufficient detail to stream-side study, mesocosms were used to quantify the relationship between nutrient exposure (nitrogen and phosphorous) and algal response as measured by growth rate and peak areal biomass. In the first year (2002), nutrient additions of N and P to reference water allowed the development of nutrient-dose response curves in the absence of complex effluents. In the second year (2003), similar nutrient concentrations were targeted using two complex effluents; municipal and pulp mill effluents. In both years, field-deployed mesocosms were used in two consecutive long-term (25 days) experiments. Seven nutrient concentrations for N (range; 25-150 μg/L) and P (range; 3-75 μg/L) were employed and were selected to allow a range of nutrient conditions (from limiting to saturating) and to simulate *in situ* conditions at relevant sites along the Wapiti River. Natural algal communities were allowed to develop on tiles placed within the mesocosms and the tiles were sampled at five-day intervals. Endpoints for the experiment included chlorophyll *a*, periphyton AFDM and algal community structure. Results indicate increasing primary production in response to increasing exposure to both N and P and suggest the existence of co-limitation involving both N and N in the upper reaches of the Wapiti River. The complex effluent mixtures in the second year contributed different relative amounts of N and P. Similar patterns in growth to the first year were demonstrated, with saturation occurring at or above current discharge levels. These experiments, combined with mesocosm experiments conducted on benthic invertebrate communities in 2001, address not only nutrient-related investigation of cause questions but also the cumulative effects of two complex effluents on the Wapiti River.

Environmental Effects Monitoring (EEM) Cycle 3 fish survey results for pulp and paper mills discharging to Lake Superior, Ontario. N. Ali, D. Audet and K. Flood. Environment Canada, Environmental Protection Branch, Downsview, ON.

Six pulp and paper mills currently discharge process effluent directly or indirectly to Lake Superior in northern Ontario. On April 1, 2004, these mills submitted their Interpretive Reports for Cycle 3 of the regulated Environmental Effects Monitoring program (EEM) to Environment Canada. Of the six mills, five of them conducted fish surveys in Cycle 3. The Cycle 3 fish survey results varied. At some sites, for certain fish species, the Cycle 3 results were consistent with Cycle 2 while at other sites, there were differences between Cycles 2 and 3 for certain measured parameters. This poster examines the results of the Cycle 3 fish studies for the Lake Superior mills and compares the results for the major EEM fish survey parameters to the results obtained in Cycle 2.

Pesticides - Pesticides

Session co-chairs: J. Hellou and W.R. Ernst

Concentrations of pesticides in water samples from St. Lawrence Estuary tributaries. M. Lebeuf[1], C.M. Couillard[1], R.L. Roy[1], C. DeBlois[2], S. Trottier[1], M. Noël[1], A. Ouellet[1] and G. Allard[1]. [1]Department of Fisheries and Oceans, Institut Maurice Lamontagne, Mont-Joli, Qc; and [2]Centre d'expertise en analyse environnementale du Québec, Sainte-Foy, Qc.

Coastal marshes of the St Lawrence Estuary (SLE) are critical spawning and feeding areas for the SLE, pesticides are widely used for agricultural and domestic (lawn care) purposes which may lead to elevated concentrations in coastal zones, particularly after storm events. The objective of this project was to obtain the first data on pesticide concentrations in rivers and streams discharging into the SLE. Water samples were analyzed for 64 non-persistent pesticides using validated methods with low detection limits (0.01-0.03 μg/L). In July and August 2002, eight south shore sites, from St. Roch-des-Aulnaies to Pointe-au-Père, were sampled. Nine pesticides were detected at low concentrations (< 1.7 μg/L). The highest concentrations of pesticides (Dicamba, Mecoprop, MCPA, 2,4-D et 2,4-DB) were found at TroisPistoles and Pointe-au-Père. Atrazine was detected at Isle-Verte and Kamouraska at levels up to 0.16 μg/L. In 2003, water from 4 sampling sites, Isle-Verte, TroisPistoles, Bic and Pointe-au-Père were sampled on 10 occasions between May-September. Eight pesticides were detected at low frequency with peak concentrations generally found early July. The most frequently detected pesticides were Simazine, Metolachlore and 2,4-D while atrazine was not detected at any of the sites. Only carbofuran was detected in our control site (Bic). This study reports low inputs of pesticides from freshwater tributaries to the coastal marshes of the south shore of the SLE. Water concentrations were below the current chronic toxicity guidelines for the protection of aquatic organisms.

Toxicity of pesticides in short-term. P.M. Jackman and K.G. Doe. Environment Canada, Environmental Science Centre, Moncton, NB.

In the past 10 years in PEI there have been 29 documented fish kills. Pesticides have been implicated as a cause in many of these fish kills because: they occur following a heavy rainfall in areas where there is evidence of erosion; there has been recent pesticide applications; the dead fish are otherwise healthy. To examine the role of pesticides in these fish kills, we conducted short term 'realistic' pulse exposures of 1, 4, and 10-h duration to mimic runoff situations. Organisms were then transferred to clean water, with follow up observations to examine delayed mortality, or recovery. The data were compared with standard test durations (96-h continuous exposure for trout, 48-h continuous exposure for *Daphnia magna*). The pesticides were tested alone, and in combination with 10,000 mg/L suspended PEI farm soil. Chemical confirmation of test concentrations was performed. The pesticides tested were Azinphos-methyl and Chlorothalonil. Results indicate that Chlorothalonil and Azinphos-methyl in 'realistic' exposures of 1, 4, and 10-h duration was significantly less toxic than toxicity in standard test durations. Interestingly, Azinphos-methyl was not lethal to *D. magna* but it caused immobilization at extremely low levels ("ecological death"). Addition of 10,000 mg/L farm soil as a suspension did not change toxicity of Chlorothalonil or Azinphos-methyl in a consistent manner. This new data should be used in risk assessments of the pesticides. Future planned research includes testing of other pesticides, comparison of short-term toxicity values to levels of pesticides measured in field runoff, and measuring the short term 'realistic' pulse toxicity of mixtures of 2 or more pesticides.

Does pesticide use in Prince Edward Island potato farms cause estrogenic responses in fish? J.P. Sherry[1], C. Tinson[1], K. Cooper[1], R. Mroz[2], W.R. Ernst[2], M.R. Servos[3] and L. Vallis[4]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, Environmental Science Centre, Moncton, NB; [3]Canadian Water Network, University of Waterloo, Waterloo, ON; and [4]Environment Canada, Environmental Protection Branch, Dartmouth, NS.

Many Prince Edward Island (PEI) streams flow through heavily cultivated watersheds where the potato is the predominant crop. Potato crops are typically given multiple applications of pesticides through the growing season. Some of the applied pesticides are suspected endocrine disrupters. We caged juvenile rainbow trout in PEI streams to test whether exposure to ambient water in streams close to potato farms could trigger an estrogenic response in fish. Because such streams are likely to receive pulsed doses of pesticides in association with rain-fall events, we sampled the caged fish 7 days after the first significant rainfall event. Induced vitellogenin (Vg), a biomarker of exposure to estrogenic substances, was measured by enzyme-linked-immunosorbent-assay (ELISA). We found no evidence of estrogenic or anti-estrogenic effects in the caged fish. We will also describe a sensitive gel electrophoresis technique for the measurement of Vg in brook trout plasma. We will report on the use of that technique to measure Vg in the plasma of feral brook trout from two of the studied rivers.

Use of confocal laser scanning microscopy for the study of vitellogenesis in Toxaphene-exposed female yellowtail flounder (*Limanda ferruginea* Storer). G.E. Fåhræus-Van Ree. Department of Biology, Memorial University of Newfoundland, St. John's, NL.

Confocal Laser Scanning Microscopy (CLSM) is a powerful tool in qualitative and quantitative demonstrations of macromolecules and physiological ions in fixed or live fluorescent specimens. This technique allows for optical sectioning in three dimensions through thick specimens, producing only in-focus images. Fluorescence may be exhibited by primary (autofluorescence), or secondary (result of added dyes and chemicals to cells or tissues), or induced fluorescence.

Vitellogenesis, the process of yolk protein formation, is crucial in the oogenesis of oviparous vertebrates. During this process, which is mediated by ovarian estrogens and controlled by the hypothalamus/pituitary system, vitellogenin, a large lipophilic yolk precursor protein, is synthesized in the liver and transported by blood circulation to the ovary. Vitellogenin enters via receptor-mediated endocytosis into the oocytes, where it is proteolytically cleaved to form yolk proteins.

The effect of Toxaphene, an environmental estrogenic contaminant, on the relative amount of yolk proteins in vitellogenic oocytes in laboratory-bred female yellowtail flounder (12.5-18 cm standard length, 39.8-100 g initial weight), was studied using the CLSM technique. Fish were untreated (initial control) or exposed to Ringers solution (blank control), corn oil and acetone (vehicle control), or Toxaphene (at concentrations of 0.2 or 2.0 μg/fish) received by a one time, weight-dependent intraperitoneal injection (Bishop, R., 2000, B.Sc. Honours thesis, Memorial University of Newfoundland, St. John's, NL). The injected fish were examined after four or eight weeks. Ovary and liver tissue sections (paraffin and frozen) were assessed for vitellogenic activity by using eosin and fluorescein isothiocyanate as fluorochromes. Hematoxylin & eosin-stained paraffin ovary sections give the best results for qualitative assessment of vitellogenic activity, while fluorescein is preferable for quantitative assessment in paraffin ovary sections and frozen liver sections. Preliminary results indicate that Toxaphene decreases the relative vitellogenic activity in the liver as well as in the ovary. Disruption of the vitellogenesis may be of great ecological significance, since it will affect reproduction and, ultimately, the population.

Depression of brain acetylcholinesterase (AChE) in fish collected downstream of agricultural activities. M.A. Gray[1], C.J. Smith[1], B.J. Park[2], V.P. Palace[2] and K.A. Kidd[3]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; and [3]Department of Biology, University of New Brunswick, St. John, NB.

The depression of acetylcholinesterase (AChE) has generally been used as an indicator of exposure to organophosphate and carbamates pesticides. A variety of small-bodied fish were collected in agricultural areas of Ontario and Manitoba in 2003 and 2004. Golden shiners (*Notemigonus crysoleucas*) were collected from 4 sites on 20 Mile Creek in southern Ontario, a stream surrounded by two separate types of farming. At the upper reaches of the stream, on the Niagara escarpment, agricultural activity is primarily based on soybean and corn production. On the lower reaches, below the escarpment, soft fruit orchards and vineyards dominate. In April 2003, shiners collected at the downstream reaches had lower AChE levels (12-18%) in brain tissue compared to the upstream sites. These differences, however, were not evident from fish collected at the same sites in July 2003. Juvenile white sucker (*Catostomus commersoni*) and common shiners (*Luxilus cornutus*) were collected at two sites in the Cypress River, in southwestern Manitoba in 2003 and 2004, respectively. This area is predominantly potato cultivation, with moderate levels of cattle farming. During at least one fish collection, both suckers and shiners showed significant reductions in AChE (29 and 45%, respectively) at sites adjacent to higher degrees of crop cultivation activities. There were no differences in terms of organ size (liver and gonad) of the fish collected in the different landuse regions. One of the main objectives of future collections will focus on establishing whether there are relationships between levels of AChE depression and responses by fish at the individual and/or population levels. Pesticide analysis will also be conducted on the fish tissues in order to attempt to make a link between exposure to pesticides and reductions in AChE activity.

Examinations of the potential for pesticides to impact golden shiners in a Niagara stream. B.J. Park[1], J. Struger[2], J.L. Parrott[3], R.E. Evans[1], K.G Wautier[1], C.L. Baron[1], K.A. Kidd[1], M.A. Gray[4] and V.P. Palace[1]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Environment Canada, Environmental Conservation Branch, Burlington, ON; [3]Environment Canada, National Water Research Institute, Burlington, ON; and [4]Department of Zoology, University of Manitoba, Winnipeg, MB.

Centre for Environmental Research on Pesticides (CERP, Fisheries and Oceans Canada) assessed potential impacts of pesticides on resident fish in Twenty Mile Creek, Ontario, Canada. The creek's drainage basin supports intensive agricultural activity, including row cropping (e.g., corn, soybean), vineyards and soft fruit production. Water samples were collected at 2 sites from April-October and screened for triazine, acetanilide and phenoxy acid herbicides and organophosphorous insecticides. Triazine and phenoxy acid herbicides were frequently detected at the two downstream sites at concentrations ranging from 85-1600 ng/L. Golden shiners (*Notemigonus crysoleucas*) were collected as a sentinel species from four sites in April, June, July and September. Histological analyses revealed no apparent differences between sites in terms of gonad development, liver cell size or thyroid gland architecture. Muscle thyroid hormone content, measured as triiodothyronine (T3) was determined for fish collected in July. Mean T3 content was significantly lower in the furthest downstream site. Brain acetylcholinesterase (AChE) activity was 12-18% lower in the brain tissues of fish from the furthest downstream sites in April, but not in July samples. Fathead full life cycle exposure studies were conducted with water taken from the furthest downstream site, and indicate that reproductive timing was affected by exposure to this water. Research on the Twenty Mile Creek system is continuing in 2004.

Pesticide science fund project - reducing pesticide impacts in aquatic systems of the Atlantic region. W.R. Ernst[1], A. Denning[1], H. Rees[2], L. Chow[2], J.M. Culp[3], K.G. Doe[4], L.M. Hewitt[5], G. Julien[4], C. Murphy[6] and J. Hellou[7]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [2]Agriculture and Agri-Food Canada, Research Branch, Fredericton, NB; [3]Environment Canada, National Water Research Institute, Fredericton, NB; [4]Environment Canada, Environmental Science Centre, Moncton, NB; [5]Environment Canada, National Water Research Institute, Burlington, ON; [6]Environment Canada , Environmental Protection Branch, Charlottetown, PE; and [7]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Potato crops in the Atlantic Region rely heavily on pesticide treatments, with an individual crop receiving up to 19 applications of various products in a single year. A high incidence of fish kills in streams draining agricultural areas has indicated a need for the development of mitigative measures. It has been generally observed that the pesticide-induced fish kills follow periods of high precipitation and to be the primary cause of the fish kills. It is necessary to know the hazards of those pesticides to aquatic organisms under realistic exposure conditions (short term for fish kills and longer term for benthic community impacts) in order to conduct comparative risk analyses to ensure that product use pattern modification can reduce overall risk. The mode of transport of individual pesticides (soluble versus adsorbed) and their bioavailability in those states is not well enough established to be able to focus on the most appropriate mitigative strategy. This project is a three year study (2003-2006) to assess the fate and transport of pesticides from potato fields in Prince Edward Island and New Brunswick and determine any appropriate management strategies.

There are six major components of the project: (1) Toxicity assessment of pulse exposures to *Daphnia magna* (Environment Canada), (2) Assessment of levels and effects of pesticides in runoff (NWRI, AAFC), (3) Ecological Assessment of benthos and algae in situ (NWRI), (4) Cultural practices assessment (AAFC), (5) Riparian buffer zone effectiveness (EC, BBEMA), and(6) Multi-media monitoring of pesticide residue concentrations (DFO, PEIDFAE). First year results, as well as future directions will be presented.

Pesticides, fish and fish habitat: a greater evaluation of risks is required. J.F. Payne. Department of Fisheries and Oceans, Science Branch, St. John's, NL.

Although the socio-economic benefits of pesticides are well documented, pesticides are inherently toxic and greater assurance should be provided that they are not posing undue risks to fish and fish habitat. As for all chemicals, the potential for risks of a chronic, hard to detect nature are of particular importance in this regard and much more emphasis needs to be placed on chronic toxicity studies. For instance, at the present time in Newfoundland, hormonal mimics such as tebufenozide or bacterial toxins such as mixtures of spinosyns, are candidate pesticides for forestry spray programs and need to be evaluated for chronic toxicity, if only for assurance. It is encouraging that endocrine disruption is presently gaining attention as an important endpoint, but immunological, neurological, and genetic endpoints are also important. It is likewise encouraging that some field studies are beginning in the Maritimes, in relation to potential effects of agricultural pesticides/herbicides on fish, and tandem laboratory and field studies for biological effects coupled with good chemical analyses should be recommended more strongly by researchers. With respect to chemical analyses, much more attention should be placed on fingerprinting metabolic by products if labile pesticides, which "bite and run" providing no evidence that they have "struck" if "evidence seeking" relies on analysis of parental compounds alone. Therefore, more investment in research is needed than presently provided, if state-of-the-art assessments are expected.

Multi component pesticide studies in the St. Lawrence River valley. L. Poissant[1], F. Aulagnier[1], C.Beauvais[1], J. Boyer[2], M. Garmouma[1] and M. Pilote[1]. [1]Environment Canada, Meteorological Service of Canada, Montréal, Qc; and [2]Environment Canada, Environmental Conservation Branch, Montréal, Qc.

Pesticides used in Québec account for up to 3,200 tonnes of active agents. Those pesticides are applied on about 1.6 millions of hectares. Moreover, about 3 kg/ha of pesticides are applied on crop other than fodder, namely on corn crops. In Québec, tonne of corn. More than 400 tonnes of pesticides are applied on the Yamaska River watershed, so called the "garden of Québec". This involves important environmental issues within this region. Many researches on pesticides are achieved into this watershed by various research groups, namely upon air, water and other ecosystem systems. Efforts are given to build ecosystem base studies on pesticides with collaboration between academic and government research groups. After presentation of the Québec pesticides issues, we will present and discuss results from various research campaigns in Québec since the nineties to recent years regarding pesticide fluxes and concentrations: in the Baie Saint François (wetlands); Saint-Anicet station (rural site); Villeroy, Mingan (remote site). It is expected that these results will allow determine the compounds mainly detected in Quebec and the evolution of the pesticide use during the last decade. Finally, we will point out the future pesticide issues to still address.

Reducing pesticide environmental risks: the National Agri-environmental Standards Initiative (NAESI). P.-Y. Caux and P.B. Jiapizian. Environment Canada, National Water Research Institute, Gatineau, QC.

The Agricultural Policy Framework (APF) is a new Federal Government program whose objective is to make Canada a world leader in food safety, innovation and environmentally responsible production. The Environment Chapter of the APF is aimed at accelerating efforts to reduce risks from agriculture and provide benefits to Canada's resources. Environment Canada is responsible for the National Agri-environmental Standards Initiative (NAESI), a program that will develop non-regulatory performance standards aimed at establishing the degree of desired environmental quality of air, water and soil in agricultural areas. This presentation will focus on NAESI pesticide activities and will frame the discussion around concepts for setting standards in the ecosystem which can be translated back to individual farms. Several activities are underway namely a prioritization scheme for pesticide standards development, a review of regional, national and international approaches for ideal and achievable standard development and their implementation, two test cases and, work linking this program to Agriculture Canada's Watershed Evaluation of BMP program (WEBs) and to the National Agri-environmental Health Analysis and Reporting Program (NAHARP). Through this work, we will increase our understanding of relationships between agriculture and the environment. Environmental standards against which we can assess the relative performance of the agriculture sector will help us determine which specific areas need improvement and further guide future research and policy development in Canada.

Concentrations of pesticides in critical zones of the Saint Lawrence Estuary and their toxicity to fish. C.M. Couillard[1], M. Lebeuf[1], R.L. Roy[1] and C. DeBlois[2]. [1]Department of Fisheries and Oceans, Institut Maurice Lamontagne, Mont-Joli, Qc; and [2]Centre d'expertise en analyse environementale du Québec, Sainte-Foy, Qc.

Coastal marshes of the St. Lawrence Estuary (SLE) are critical habitats for several fish species. They are potentially contaminated by pesticides from urban or agricultural runoffs. Our objectives were: (1) to measure concentrations of pesticides in SLE tributaries irrigating 4 coastal marshes, (2) to evaluate fish health and biomarker responses in resident threespine stickleback (*Gasterosteus aculeatus*), and (3) to determine toxicity thresholds of selected pesticides for marine fish larvae. From May-September 2003, water was sampled weekly at each site. Concentrations of pesticides were generally low, but were higher at agricultural sites (IleVerte, Trois-Pistoles) than at Pointe-au-Père, an urban site, or Bic, the reference site. The herbicide atrazine (≤ 200 ng/L) and the insecticide diazinon (≤10 ng/L) were detected and selected for toxicological evaluation. In June-July 2003, sticklebacks at the 4 sites were sampled on three different occasions. The condition factor, the gonadosomatic index and the activity of acetylcholinesterase (AChE) in muscle did not differ among sites. Finally, mummichog (*Fundulus heteroclitus*) larvae were exposed experimentally to diazinon during 96-h. Mummichog growth was inhibited at the lowest exposure concentration (124 ng/L), while inhibition of AChE was observed only at concentrations ≥ 361 ng/L. These results indicate that inputs of pesticides from the SLE tributaries to the coastal marshes are relatively low and that there are no obvious toxic impacts in resident fish. Further studies are underway to evaluate pesticide inputs from the marine water of the SLE and to investigate potential endocrine disruption in these fish populations.

Residues of organochlorine pesticides in farm areas of the lower Fraser Valley, British Columbia, Canada. J.-N. Kuo, M.T. Wan and J. Pasternak. Environment Canada, Environmental Protection Branch, Vancouver, BC.

Crop soils, ditch sediments and water flowing from several farm areas to salmon tributary streams of the Fraser River in the Lower Fraser Valley (LFV) of British Columbia, Canada, were sampled in 2002-2003 to quantify residues of selected organochlorine (OC) pesticides. Reference and background levels of these pesticides were obtained from pristine watershed areas. Current concentrations of OC pesticides in soils of the LFV reported by Szeto and Price (1991), Wan (1989) and Wan et al. (1995) were used for comparison. Varying amounts of aldrin, benzene hexachloride (BHC), chlordane, dieldrin, endrin, heptachlor, methoxychlor, p,p-DDT and their respective major transformation products were detected, although their use has been discontinued since early 1970s. In 2002/2003, the mean total OC pesticide residues, excluding endosulfan (END), in crop soils of the LFV appeared to decrease by about 51.5% to an estimated 3.630 mg/kg d.w. when compared with that found in 1991.

Szeto, S.Y., and Price, P.M. 1991. Persistence of pesticide residues in mineral and organic soils in the Fraser Valley of British Columbia. Agric. Food Chem. **39**: 1679-1684.

Wan, M.T. 1989. Levels of selected pesticides in farm ditches leading to rivers in the Lower mainland of British Columbia. J. Environ. Sci. Health **B24**: 183-203.

Wan, M.T., Szeto, S.Y., and Price, P. 1995. Distribution of endosulfan residues in the drainage waterways of the Lower Fraser Valley of British Columbia. J. Environ. Sci. Health **B30**: 401-433.

Preliminary analyses of organochlorine pesticide residues in sport fish from Lake Erie. G. Tomasino, R. Grebenok, P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Organochlorine pesticides (OC's) are persistent organic pollutants that undergo bioaccumulation throughout the food chain, and hence represent potential threats to human health via consumption of contaminated food. Several studies have monitored OC pesticide levels in fish from the Great Lakes, but data for Lake Erie fish have been minimal. Trout (5), smallmouth bass (7), and yellow perch (7) were collected in the summer of 2003 from Lake Erie. Skin-off, trimmed fillets (muscle only) were extracted, interferents removed, and analyzed using EPA methods 3540C, 3620B, and 8081B respectively. Lipids were removed using a fumed silica washing step prior to florisil cleanup. OC pesticides were expressed as mg/kg wet tissue weight. Total levels of OC's were highest in the trout (633±256 mg/kg), followed by the perch (278±162 mg/kg), and bass (265±258 mg/kg). All fish examined contained OC pesticides, with preliminary identification of residues, based on retention times, indicating the presence of DDT derivatives, BHC, chlordane, and endosulfan. GC-MS confirmation of these residues is underway. Levels of individual residues do not exceed EPA recommended levels for human consumption based on one meal per month.

An assessment of the effectiveness of 10 m buffer zones in minimizing pesticide runoff from potato fields in Prince Edward Island – 2003 results. Denning[1], A. Cook[2], K.G. Doe[2], W.R. Ernst[1] and G. Julien[2]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; and [2]Environment Canada, Environmental Conservation Branch, Moncton, NB.

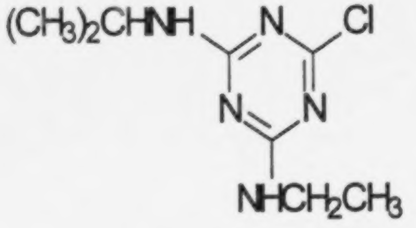
Environment Canada has been studying the effectiveness of ten metre grassed buffer zones adjacent to potato fields in reducing pesticide concentrations and toxicity in Prince Edward Island (PEI) since 2001. Provincial legislation requires a 10 m buffer between potato fields and watercourses in order to decrease the amounts of particulates, nutrients, pesticides and organic matter entering waterways across PEI. This requirement was in response to the increased number of pesticide-induced fish kills that have occurred in PEI during the past 10 years. The goal of this study is to measure pesticide and nutrient concentrations and toxicity of runoff from potato fields prior to and after traversing buffer zones of different widths following heavy rainfall events. Surface runoff is collected at the edge of the field and also at a collection unit 10 m down slope of the field (and in several fields at greater distances) in the grassed buffer. Samples are analysed for general water chemistry, pesticide concentrations and also toxicity to *Daphnia magna*, a freshwater flea. The 2001-2002 results were presented at the 2003 Aquatic Toxicity Workshop in Ottawa, ON. The poster provides an update of the results to include the data collected in 2003. In 2003, twelve fields were selected for runoff sample collection at 0 m, 10 m, (and in one field at 20 m and in another field at 30 m), with a total of seven runoff events. The results from 2003 are similar to those from the previous years, indicating that the 10 m buffer is generally effective at reducing pesticide concentrations but not as effective in reducing the toxicity of the runoff.

Pesticides in water and surface sediments of an estuary on Prince Edward Island, Canada. J. Hellou[1], A. Cook[2], W.R. Ernst[3], J. Leonard[1] and S. Steller[1]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [2]Environment Canada, Environmental Science Centre, Moncton, NB; [3]Environment Canada, Environmental Protection Branch, Dartmouth, NS.

Interest in the fate and effects of pesticides used in agriculture represents a new field of research for the Bedford Institute of Oceanography that was initiated because of a Memorandum of Understanding signed by the Department of Fisheries and Oceans (DFO) and Health Canada. The Maritimes region combined its efforts to address important questions raised in Prince Edward Island (PEI) where a history of fish kills was linked to the use of pesticides. Scientists from the Marine Environmental Sciences Division are participating in a project led by a scientist from the Gulf Fisheries Centre, Dr. Wayne Fairchild. Therefore, three DFO labs are combining their efforts, to study additional aspects of a larger undertaking by investigators in Environment Canada and the government of Prince Edward Island. Drs. Kats Haya and Les Burridge from the St. Andrews Biological Station, and Dr. Jocelyne Hellou from the Bedford Institute of Oceanography, as well as Dr. Wayne Fairchild are studying potential biological effects of pesticides on marine organisms.

Pesticides such as azinphos-methyl and chlorothalonil have been linked to fish kills in recent years (Fig. 1). Azinphos-methyl, also sold under the name of Guthion®, is an insecticide used to control leaf-feeding insects, such as the potato beetle and the corn borer (Kamrin 1997). Chlorothalonil is a widely used fungicide that is used to control fungal diseases such as late blight and downy mildew (Environment Canada, 2004). In many cases, pesticide-laden runoff can be attributed to fish kills shortly after a pesticide application and following rainfall events. It is noteworthy to mention that while "fish kills" may be the most obvious consequence of such pesticide problems, pesticides kill far more than fish and the effects of contamination on an ecosystem as a whole should be considered.

Azinphos-methyl



Chlorothalonil

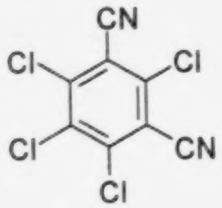


Fig. 1. Structure of two pesticides involved in fish kills.

Pesticide	Water (ug/L)	Sediment (ug/g)
Atrazine	<0.008	<0.02
Azinphos-methyl	<0.038	<0.04
Azoxystrobin	<0.039	<0.08
Carbaryl	<0.012	<0.03
Carbofuran	<0.007	<0.03
Chlorothalonil	<0.005	<0.08
Cypermethrin	<0.028	<0.07
Dimethoate	<0.021	<0.02
α-endosulfan	<0.007	<0.06
β-endosulfan	<0.008	<0.06
Fonofos	<0.008	<0.04
Heptachlor epoxide	<0.007	<0.06
Hexazinone	<0.043	<0.02
Linuron	<0.016	<0.03
Metalaxyl	<0.028	<0.02
Metobromuron	<0.013	<0.05
Metribuzin	<0.012	<0.06
Permethrin	<0.030	<0.07

Table 1. With one exception (not listed abov)e, the 18 pesticides were below detection in both water and sediment samples; the limits of detection are expressed in the table.

Pesticide use is a mainstay to the agriculture industry and as such has become vital to the province of PEI as a whole. Potatoes alone are the largest agricultural commodity in the province, which typically have an annual farm value nearing 200 million dollars, and account for over fifty percent of total farm cash receipts (PEI Department of Agriculture, Fisheries, Aquaculture and Forestry, 2004). Overall, primary agriculture and related agri-food processing contributes eleven percent to the provincial Gross Domestic Product (PEI Department of Agriculture, Fisheries, Aquaculture and Forestry, 2004). With such importance placed on the industry in terms of the province's economic health, the merits of the use of pesticides and the needs of farmers should not be overlooked.

Within DFO, concern for levels of contaminants in organisms has traditionally focused on human health guidelines for the suitability of fishery products as food. This perspective has progressed with time, to include a more ecological outlook. Assessing the health of various species, including effects of contaminants, has become important for both the maintenance of a sustainable environment and protection of fishery resources.

This publication presents the results from the first year of a three year project (2003-2006) investigating the levels of a series of pesticides suspected to be in the marine environment, as a result of land application. To complement research activities of federal and provincial colleagues in Environment Canada and PEI, respectively, sampling took place in the Wilmot River and Dunk River estuary of Summerside Harbour in PEI (Fig. 2). The city of Summerside is located approximately fifty kilometres west of Charlottetown and is of interest due to previously encountered fish kills related to pesticide applications near rivers leading into Summerside Harbour. The estuary itself receives input from the Wilmot River, draining effluents from a number of farms undergoing pesticide remediation experiments, and from the Dunk River, representing more of a reference site where remediation has not been attempted.

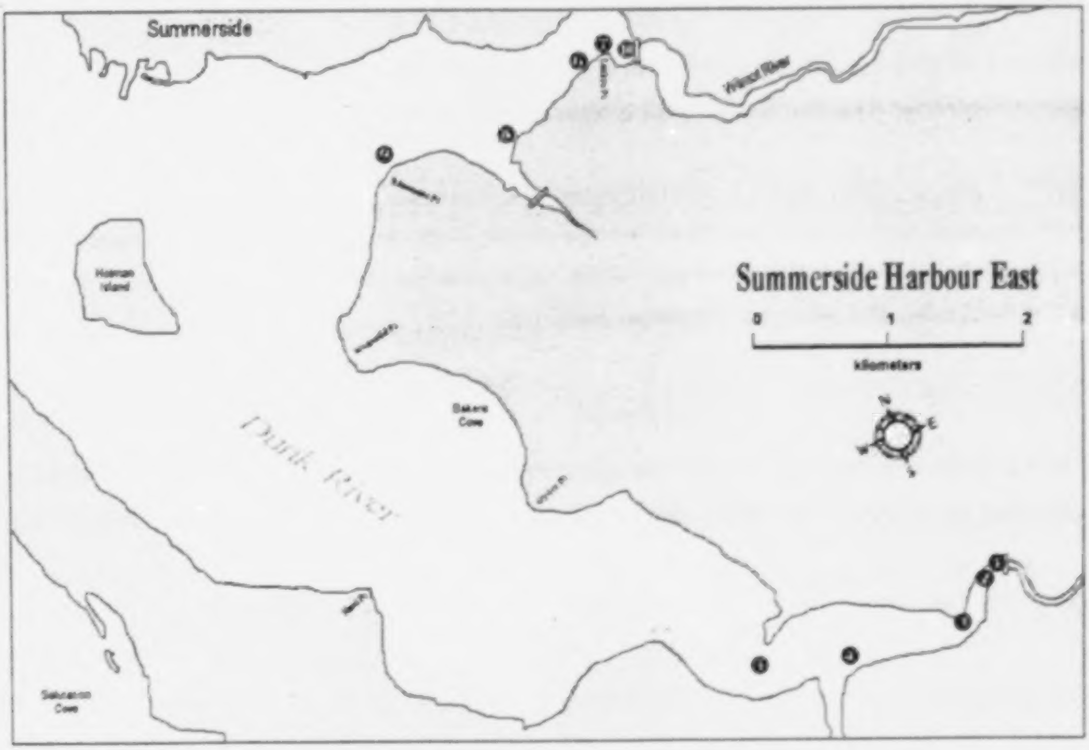


Fig. 2. Samples were collected from 5 sites located at approximate distances of 1, 100, 500, and 2000 m from the outermost bridges of the Dunk River and Wilmot Rivers.

It is known that pesticides can bind to soil and be transported in particulate and soluble phases. As such, pesticides could be transported from the locations where they are applied, swept into nearby rivers, and then end up in estuaries like the Summerside Harbour estuary. As it is known that pesticides have been involved in a number of fish kills that took place over the past few years on Prince Edward Island, the degree of pesticide contamination in the estuary is of interest. A list comprised of eighteen pesticides, ranked by the Air and Toxics Issues Section of Environment Canada Atlantic according to use in PEI, bioconcentration potential, and known toxicity, were analysed in a number of water and sediment estuarine samples (Dunn 2004).

Surface sediments were collected with an Eckman grab sampler and placed in pre-cleaned glass mason jars with lids lined with solvent rinsed aluminium foil. Samples were placed on ice within 2 h of collection and frozen within 24 h. Water was collected at a 10 cm depth in pre-cleaned 1 L amber glass bottles. Samples were collected from 5 sites located at approximate distances of 1, 100, 500, 1000, 2000 m from the Dunk and Wilmot Rivers (Fig. 2). The first sample set of sediment and water samples (collected from the total of ten sampling locations) was collected 24 h after the beginning of a rainfall event on August 8, 2003. Sample collections at the ten sites were later repeated on September 11, 2003 and October 23, 2003. Water temperature (8-25° C) and salinity (11-28‰) were determined, along with total organic carbon content (0.6-4.1%) of sediments.

The eighteen pesticides were not detected in either the water samples or in the sediment samples, with the exception of chlorothalonil (0.6 mg/kg at site 4 on the Dunk River in October). To improve detection in the water samples, the samples from five sites along a river (that were sampled at one point in time) were combined and the volume reduced before GC-MS analysis. Although detection limits were improved by a factor of 20, pesticides were still not detected. Further analyses of samples are currently underway to determine trace pesticide concentrations of a subset of pesticides that may be detectable through more complex analytical procedures.

Results will be used to place the following two years study of potential sub-lethal toxic effects of some pesticides on selected marine organisms, chosen for their commercial or ecological value, in an environmental context.

Acknowledgements

This is a companion investigation to a larger study with Environment Canada and the Province of PEI. Credits: B. Bema, L. Chow, A. Cook, J. Culp, A. Denning, K. Doe, L. Edwards, B. Ernst, C. Garron, M. Hewitt, G. Julien, J. Mutch, C. Murphy, S. Murtagh, H. Rees, and K. Webb. We would like to thank our collaborators in this first year of the study Mr. Lea Murphy, Deryck Mills, Delly Keen, John McKinnon, and Danny Geldert at DFO, PEI who facilitated our sampling in the Wilmot and Dunk Rivers, by providing a boat, GPS system, sampling experience and enthusiasm. We also acknowledge funding by the Department of Fisheries and Oceans (DFO) National Pesticide Research Fund (NPRF).

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How to improve the environmental realism of aquatic pond mesocosms for regulatory decisions on pesticide registration? E.M. Foekema. Netherlands Organization for Applied Scientific Research; Environment, Energy and Process Innovation; Den Helder, Netherlands.

For the registration of crop-protecting products, authorities can request aquatic model-ecosystem studies when questions about the environmental impact of the product are not answered by first tier laboratory tests. The aim is to assess the Acceptable Ecological Concentration by investigating the impact of the product on an aquatic ecosystem under more realistic environmental conditions than in the laboratory. Lentic pond mesocosms are amongst the most commonly used model-ecosystems for this purpose. They are often recognized as representing an extreme worst case situation because of the lack of water exchange, resulting in maximum exposure concentrations, limited recolonization of (initially) affected species, and nutrient depletion that could affect the recovery of phytoplankton and macrophytes. These worst-case conditions may result in an overprotection of the actual field situation, and are therefore often the point at issue in discussions between pesticide producers and regulatory authorities. Examples from TNO's mesocosm studies for regulatory purposes will be presented to demonstrate the possible impact of these worst-case conditions on the determination of the Acceptable Ecological Concentration. Suggestions are presented for increasing the environmental relevance of pond mesocosms by including some water exchange, improved nutrient availability and the possibility for recolonization of the affected area. We challenge both improve the realism of experimental pond mesocosms.

Effect of exposure duration and dissolved oxygen condition on chronic imidacloprid toxicity to ***Chironomus tentans*****.** S.J. Stoughton[1], K. Liber[1], J.M. Culp[2] and A. Cessna[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water research Institute, Fredericton, NB; and [3]Environment Canada, National Water Research Institute, Saskatoon, SK.

Imidacloprid is a relatively new nicotine mimic insecticide that is widely used across North America. Imidacloprid is relatively water-soluble and thus is subject to runoff. Residues are therefore routinely found in surface waters near treated fields, yet little is known about chronic effects of this insecticide on non-target aquatic biota under realistic environmental conditions. The following research was aimed at addressing these data gaps. The toxicity of imidacloprid to the invertebrate, *Chironomus tentans*, was first evaluated in 96-h tests using both technical material (99.2% pure) and Admire®, a formulated product (240 g/L a.i.). The 96-h LC50s for Admire® and technical imidacloprid were 1.18 and 1.40 μg/L. Based on these data, Admire® was chosen for a 28-d static-renewal test starting with 7-d old *C. tentans*. Exposure scenarios consisted of constant press (28-day) and pulse (4-d, followed by 24-day in clean water) exposure regimes. For the press exposure, larval growth, survival, and adult emergence decreased at 3 μg/L, but not at 1 μg/L. There was no effect at 3 μg/L in the pulse exposure. Therefore, chronic (≥ 28 day) imidacloprid exposure may result in adverse effects, but organisms can tolerate short-term (≤ 4-d) exposure to similar imidacloprid concentrations if organisms are then moved to clean water. A similar 28-d test with *C. tentans* is currently being run under both high (6 μg/L) and low (1 μg/L; sublethal) dissolved oxygen conditions, to evaluate whether confounding low dissolved oxygen stress (which can co-occur with pesticide and nutrient loading to water bodies) will enhance the chronic toxicity of imidacloprid.

Effects of the insecticide imidacloprid on ***Epeorus longimanus*** **and** ***Lumbriculus variegatus*** **feeding and growth.** A. Alexander[1], J.M. Culp[2] and D. Baird[2]. [1]Department of Biology, University of New Brunswick, Fredericton, NB; and [2]Environment Canada, National Water Research Institute, Fredericton, NB.

Imidacloprid is a highly soluble and persistent insecticide that targets aphids, whiteflies and the

Colorado potato beetle and may accumulate in the aquatic environment (Moffat 1993, Julien *et al.* 1996, Sarkar *et al.* 1999). This insecticide was first identified as a threat in France where the toxicity of this compound to bees was first noted (CHC, Suchail *et al.* 2001). Recent research identifies several aquatic invertebrates (i.e. mayflies and oligochaetes) as the most negatively affected lotic species (Culp *et al.* unpublished data). Because of the potential non-target threat to aquatic invertebrates, I will compare the effects of the insecticide imidacloprid on the feeding, growth and abundance of the aquatic grazer, the mayfly *Epeorus longimanus*, and a deposit feeding oligochaete, *Lumbriculus variegatus*. Using pulse exposures, the sublethal impacts of imidacloprid will be measured through short-term effects on feeding rate and longer term effects on growth. Pulse exposures are employed as a realistic exposure scenario because pulses are generated by agricultural field run-off after heavy rain events. Subsequent laboratory work will use the range of concentrations surrounding the lowest effect concentration (LOEC) to examine the long term effects of imidacloprid on growth of mayflies. The laboratory experiments will describe the magnitude and duration of the effect of imidacloprid on the two functional feeding groups. The project employs a tiered approach using laboratory, mesocosm and field experiments.

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Ecological risk assessment / Évaluation des risques écologiques

Session co-chairs: C.E. Moore and U. Klee

Reducing uncertainty in Environmental Impact Assessment (EIA) by integration of Environmental Effect Monitoring (EEM) and Environmental Risk Assessment (ERA). M. Smit, C. Karman and J. van Dalfsen. Netherlands Organization for Applied Scientific Research, Environment, Energy and Process Innovation, Den Helder, The Netherlands.

For the evaluation of impacts in the aquatic environment many tools for environmental impact assessment (EIA) are available. In general these tools can be divided into two categories: environmental effect monitoring (EEM), and environmental risk assessment (ERA). Although both tools have the same assessment endpoint (identifying the severity and extend of biological effects) up to now not much effort is put in the integration of both tools. Regulators and industry consider both methodologies but the results of EEM studies and ERA models have not been, or cannot be, compared in a general scientific-sound way, mainly due to missing links between the two methodologies. A framework for EIA wherein EEM and ERA are integrated can be profitable in many ways. Analysis of the assessment endpoints of ERA and EEM indicate that integration compensates the uncertainties in EEM by the strengths of ERA and vice versa. This paper presents an integrated framework for EIA as a basis for policy making and definition of management tools. Necessary developments in ERA and EEM for a better integration will be defined and discussed. Examples from different fields will be given to illustrate how ERA and EEM can support each other, reduce uncertainties and improve EIA.

Null variables in environmental monitoring. M.D. Paine. Paine, Ledge and Associates, North Vancouver, BC.

Environmental effects monitoring (EEM) use Reference stations or areas as a spatial "Control," and baseline (pre-operational or Before) data may be used as a temporal "Control." These spatial and temporal controls are not always available, and another option may be Control or Null variables. Null variables are easy to find for freshwater to marine discharges, because concentrations of many metals and elements are naturally greater in marine waters. Sodium and magnesium concentrations in sea water should never be affected by freshwater discharges. Aluminum (sand is aluminum silicate) and iron are good null variables for marine sediments. Strontium is a good null variable for tissue, since it is used as an indicator of marine residence for anadromous fish. The use and value of null variables will be illustrated with case studies. When "effects" (spatial differences; temporal trends or other time differences) are detected for null variables, that provides an empirical demonstration of power (you were able to detect subtle natural effects, so were presumably able to detect project effects) and perspective (how big are project effects relative to natural effects?) Cu up 5% and Al up 10%? Not to worry; the copper increases are probably an analytical artifact (improved recoveries) or a natural geochemical change or difference. Al down 5% and Cu up 10%? Start worrying about copper, even if the increase is not statistically significant. It may be more difficult to find chemical null variables for freshwater to freshwater discharges and for biological effects versus chemical variables.

Receiving environment monitoring program for Lions Gate Wastewater Treatment Plant, Burrard Inlet, BC: a program under development. F. Bishay, S.D. St-Jean and P. van Poppelen. Greater Vancouver Regional District, Burnaby, BC.

The Greater Vancouver Regional District is committed to a receiving environment monitoring approach to managing its liquid waste discharges in its Stage 2 Liquid Waste Management Plan. Such a monitoring approach for the receiving environment at Lions Gate Wastewater Treatment Plant (WWTP) is currently under development. The Lions Gate WWTP, a primary treatment plant, serves a population of about 160,000, and an area of more than 8,760 ha with an average annual flow of 92 million liters per day in 2003. The effluent is discharged into Burrard Inlet at First Narrows, and the plume disperses either east or west into the inner harbour or outer Burrard Inlet depending on the tide. An approach that relies on receiving environment monitoring must be capable of detecting adverse effects before significant environmental impacts occur. As such, appropriate monitoring programs are required to define the nature, severity and extent of environmental effects of a discharge. Dispersion modelling as well as various imaging surveys were conducted to identify the potential zones of influence and delineate the appropriate study area. Based on this work and initial sediment survey studies (including samples for chemistry and benthic invertebrate), the need for additional tools to eliminate confounding factors resulting from the heterogeneous nature of the habitat (i.e., cobble near-field and silt far-field) and likely subtle effects due to the dynamic and well mixed nature of the immediate receiving environment was recognized. The results from the sediment surveys and caged bivalve studies will be reviewed, and the challenges, and future direction of this developing REM program will be discussed.

Development of a Greater Vancouver Regional District trigger process for the protection of the receiving environment. A. van Roodselaar[1], B. Burd[2], A. Lewis[1], P. van Poppelen[1], F. Bishay[1] and S. Bertold[1]. [1]Greater Vancouver Regional District, Burnaby, BC; and [2]Ecostat Research Ltd.

Abstract

The Greater Vancouver Regional District (GVRD) has committed to management of liquid waste in a sustainable and cost effective manner that protects and enhances the receiving environment. This commitment is detailed in the District's Liquid Waste Management Plan (LWMP), mandated by the Province of British Columbia, and in the Cautions, Warnings and Triggers document submitted to the province in January 2004. The development of this process will be described in this presentation.

There are five wastewater treatment plants (WWTP) in the GVRD. Environmental monitoring conducted by the GVRD will determine environmental risk, and respond in pre-defined ways for three environmental compartments: water column, sediment and biota. Criteria for the selection of cautions, warnings and triggers include but are not limited to existing guidelines such as applicable Water Quality Objectives and Canadian Council of Ministers of the Environment guidelines. In order to set a basis for comparison, the temporal and spatial scale of variability expected in background "present-day" conditions must be determined. In addition to the regular monitoring programs a variety of extraordinary studies are taking place. The adaptive and dynamic nature of the "cautions, warnings and triggers" process allows flexibility necessary for the introduction of relevant new information.

Keywords: triggers, warnings, biota, water column, sediment, benthos.

Introduction

The Greater Vancouver Regional District (GVRD) has committed to the principle of managing liquid waste in a sustainable and cost effective manner that protects and enhances the receiving environment. This commitment is detailed in the District's Liquid Waste Management Plan (LWMP). Upon approval of the LWMP, the Minister of Water, Lands and Air Protection (WLAP) required that the GVRD "Develop the environmental 'triggers' used in the monitoring process by January 31, 2004, recognizing that the environmental monitoring process in the LWMP is based on discharge indicator trend analysis such that action will be implemented before Water Quality Objectives or other criteria are met or exceeded".

Following various workshops and a multitude of special studies, as well as an expansion of the routine monitoring programs, a framework was developed to meet both the Minister's requirement and the GVRD's needs to be able to manage its aquatic environment. The framework forms the foundation of a process for indicating ecological changes prior to adverse environmental effects occurring due to GVRD liquid waste discharges to the receiving environment. This framework and the current application of detailed components, were outlined in a document titled "Cautions, Warnings and Triggers: a Process for Protection of the Receiving Environment" (GVRD 2004).

The cautions, warnings and triggers framework is primarily a weight-of-evidence approach. Indicators have been selected for different environmental compartments. Based on the level of the indicator outcomes, certain responses are defined. Options for managing the defined responses are developed by the District and member municipalities and assessed according to ecological, social and economic criteria.

The adopted concept of "management of the environment" requires that management tools are open and responsive to change. This change can be societal, environmental, or simply advances in scientific knowledge. The adaptive and dynamic nature of this framework provides the flexibility necessary for the introduction of new and relevant information that may develop through other studies and initiatives. It is also anticipated that as the scientific knowledge develops the process will evolve to accommodate this new science pertaining to sensitive monitoring tools, persistence and environmental fate of contaminants of concern, level and type of risk expressed by certain chemicals in the environment, mode of transport within the food chain, interactions within the environment, and the identification of emerging issues. A process such as this is an open process that must be sensitive to the changing scope of scientific knowledge and consequently be dynamic and adaptive in nature.

This paper aims to provide an overview of some of the considerations, tools and strategies that were developed in order to construct the framework document and facilitate its implementation (GVRD, 2004).

This framework document (GVRD 2004) provides a full discussion of the application of the cautions, warnings and triggers approach to each of three primary compartments of the aquatic environment, including: (1) Water Column – (Chapter 2); (2) Sediment – (Chapter 3); (3) Biota – (Chapters 4 and 5); (a) Benthos – (Chapter 4); and (b) Higher trophic levels – (Chapter 5). A copy of the full document may be obtained by contacting A. van Roodselaar.

The Trigger Process

The cautions, warnings and triggers approach is applied to the three compartments of the aquatic environment: water column, sediment and biota. The latter includes higher trophic levels which may be indirectly related to the specific water body and may be subject to a number of influences and factors.

Appropriate criteria for the selection of cautions, warnings and triggers include but are not limited to existing guidelines such as BC Water Quality Objectives (WQO) or Canadian Council of Ministers of the Environment (CCME) guidelines, toxicity data and statistical variability. The advantage of Objectives and Guidelines is that "fixed numbers" are available for a number of substances (not all possible substances) which must be or should be met, regardless of the state of an ecosystem. The disadvantage is clearly that non-referenced substances or unique local situations may have an effect on the local ecosystem but are not taken into account if the Objectives and Guidelines numbers are the sole criteria to be adhered to.

The most common approach to defining the state of an ecosystem (and therefore ecosystem health) is to measure the current state as some proportional deviation from "normal" or "reference" conditions. Therefore, in order to set a measurable standard, the temporal and spatial scale of variability expected in background "present-day" conditions must be known. This information is generally derived from the monitoring of defined reference areas. However reference areas are themselves influenced by a number of factors, either short term or longer term, examples of which are season, large scale or global influences such as El Nino, and in the case of ecosystems, population dynamics which lead to expansion or contraction of specific inhabitants of the area. Therefore simple nomination of an area as reference area is insufficient but should be interpreted in terms of the larger overall ambient environment. This requires, as well as an immediate effect monitoring zone and reference area program, the continuous availability of data on change in ambient conditions. If such data is not available, the institution of an "Ambient Monitoring Program", looking at broader change of conditions is required. For this reason the GVRD has developed, in cooperation with others, Ambient Programs for the Fraser River, Burrard Inlet and the Georgia Strait. Within the framework as designed a number of terms have been allocated a specific meaning and these include: exposure indicators, cautions, warnings triggers and regional cautions.

Exposure Indicators

Exposure indicators provide a context of the spatial extent and magnitude of receiving environment exposure to the discharges. The selection of indicators depends on a number of factors including reference ranges, overall consistency, potential for influence from confounding factors, ecological relevance to ecosystem health and overall recognition by other jurisdictions. Cautions, warnings and triggers must therefore be based on selected exposure indicators.

"Regional cautions" are only applied to the framework in a general sense, and are used to illustrate that a certain concern has been detected in the environment. These indicators are representative of ecosystem health but do not lend themselves to direct linkage to the discharge. An example would be fish and mammal health. A detected change is inherently important and may instigate further investigation, but cannot be attributed to any specific source.

"Cautions" occur when spatial and/or temporal changes have occurred in ambient or reference conditions outside a pre-determined margin set by sampling methodology. Cautions provide a means of continuously assessing the performance of reference levels for indicators, for recognizing unexpected (natural and anthropogenic) influences external to the discharge, changes in methodology for monitoring programs or long-term natural environmental or biological cycles. The ongoing Ambient Monitoring Programs in the Strait of Georgia and the Fraser River provide further validation of reference ranges.

"Warnings" indicate change in substance or biota levels of potential ecological concern relative to the current condition of the given receiving environment, which can reasonably be attributed to the discharge in question. Exceedance of a warning level initiates intensified sampling and monitoring to confirm cause, predict progression towards trigger status and assess the potential need for actions to slow, reverse or stop the observed trend.

"Triggers" are based on negative changes beyond warning levels in the receiving environment, ultimately showing a trend towards environmental degradation in the future and therefore harboring pressing ecological imperative. Trigger levels will ultimately prompt some mitigating action, which may have far-reaching societal, environmental and economic implications.

It is important to recognize that, in particular in the case of “warnings” and "triggers", the aim is to detect *trends*, not specific, isolated and one-off events. Should the *trend* indicate progression to an undesirable status, then some action is determined which can be taken before significant change occurs or is irreversible.

Indicators, cautions, warnings and triggers in the water column

Indicator substances used to assess achievement of water column caution, warning and trigger levels include conventional constituents, metals and trace organics. The status of indicator substances is determined by comparison with selected levels. These levels are based on Provincial Water Quality Guidelines (PWQG) and site-specific Water Quality Objectives (WQO). Therefore they vary from site to site. In addition not all substances listed in this manner are suitable for use as indicators. Therefore a distinction is made between "full" and "partial" indicators.

Full indicators are defined as substances that: (1) have an associated PWQG; (2) are monitored in GVRD wastewater effluents and the ambient (background) environment; (3) are expected to be found at measurable levels in GVRD wastewater effluents; (4) have background levels that are lower than established PWQG and WQO; (5) have an analytical detection limit that is lower than the PWQG and WQO; and, (6) do not have confounding influences that make it difficult to determine the contribution of the liquid waste discharge to the concentration at the IDZ boundary.

Partial indicators for WWTPs and CSOs are substances monitored in the effluent that have no background or ambient data or have analytical detection limits higher than either the maximum or average PWQG but not both. In simple terms, if any of the monitored substances do not meet all the criteria for Full Indicators, they are used as Partial Indicators. For substances without background data, the background concentration will be assumed zero until data are obtained.

Indicators assume caution, warning or trigger status depending on specific numerical criteria as summarized below:

Water Column Caution Levels: substance concentration outside the Initial Dilution Zone is greater than or equal to 60% of the relevant Provincial Water Quality Guideline value;

Water Column Warning Levels: calculated substance concentration at the Initial Dilution Zone boundary is greater than or equal to 60% of the relevant Water Quality Objective value;

Water Column Trigger Levels: measured substance concentration at the Initial Dilution Zone boundary is greater than or equal to 80% of the relevant Water Quality Objective value.

Indicators, cautions, warnings and triggers in sediment

The approach to defining sediment cautions, warning and triggers is based on the requirement to correlate sediment data with biotic information. Further guidance on the combined application of sediment and biotic cautions, warnings and triggers is given in the benthic section. Where provincial site-specific Water Quality Objectives (WQO) for sediments apply, these values are included in the trigger process. In BC, the provincial Approved Water Quality Guidelines (AWQG) are predominantly associated with water column values. The Working Water Quality Guidelines (WWQG) sediment-associated values do not enjoy the same confidence or acceptability status as the AWQG, and the Ministry advises that caution should be exercised in the application of these guidelines.

The use of a Sediment Quality Index has been shown to be a reasonable sediment indicator sensitive to changes in sediment quality and has been developed as an appropriate indicator tool. It has been demonstrated that the Index can be used to track temporal and spatial trends. These trends can then be correlated with benthic health data to determine the relevance of, and contribution from, a given contaminant source.

Provincial Water Quality Guidelines and site-specific WQO will be used in assessing sediment quality in calculating the Sediment Quality Indices. These index values will be used to identify a caution (guidelines) or warning (objectives) status only, because exceedance or attainment of guidelines or objectives does not necessarily indicate either an effect or the lack of an effect. Nor are sediment data automatically directly attributable to certain discharges. Certain metals are naturally high in the ambient environment and, in any case, may vary temporally or spatially. Effects, or the lack of these, can only be determined by biota surveys which are directly indicative of the health of the environment. A weight-of-evidence approach is thereby adopted.

Sediment Quality Index values are calculated annually for individual and groups of benthic monitoring stations. Index values are then compared to a caution or warning level that is based on a range of two standard deviations around an average annual Sediment Quality Index value. If the caution or warning level were to be exceeded on any one or multiple stations, the substances comprising the index would be analyzed to identify any specific substances causing the increase in the index. Should the subsequent year's sampling program identify a further or sustained increase in the index values compared to the applicable caution or warning levels, an investigation identifying the cause of the increase would be carried out including assessment of its level of attribution to the potential contaminant source.

Indicators, cautions, warnings and triggers in benthos

The approach to defining cautions, warnings and triggers in the benthos involves defining ecologically and statistically relevant departures from background or existing biotic conditions, since there are no widely accepted biotic guidelines for benthos. Only the marine outfalls are currently considered, since an on-going benthic biological monitoring program is not in place for the other discharges.

Biotic indicators integrate the long-term, chronic and acute effects of effluent exposure from wastewater discharges. The marine benthos encompasses a range of organism sizes and trophic levels which are the most direct targets for the settlement of particulate materials from municipal liquid waste discharges. The infaunal benthos tend to be numerous and diverse in marine sediments, and thus amenable to statistical analyses of distributional patterns. Most species have a life span of 1-5 years, and spawn annually, making changes in distribution patterns of the fauna interpretable in an annual monitoring program.

Separate reports, listed and referenced in the main document as well as included in the appendices described issues and questions which had to be addressed during the exposure, warning or trigger indicators selection process, as well as preliminary tasks which were required to develop benthic cautions, warnings and triggers for the Iona and Lions Gate receiving environments. Tasks included a review of indicators and triggers from other jurisdictions and an ecological significance assessment of the monitoring data for Iona from 2000 to 2003. Because the IONA monitoring program is the more long-term and advanced of the two (the other being Lions Gate), with clear-cut sediment deposition patterns related to the outfall, the development of cautions, warnings and triggers was based on this data.

As a result of the analysis of data obtained from the longest running GVRD monitoring program, the following effect zones were determined as occurring along a gradient from a particular outfall and useful as a general qualitative description for various situations and locations:

Moderately impacted (MI): moderate declines in abundance, species richness, biomass and the most abundant and relatively tolerant bivalve, *Axinopsida serricata*; virtual elimination of echinoderms, crustaceans, modest enrichment of opportunistic polychaetes and dominance of a few taxa (decreased Swartz Dominance Index - SDI); highest AVS, sterol (i.e. coprostanol) and nonylphenol levels. Conditions suggest reduced oxygen in sediments. This effect zone fluctuates in the vicinity just north of the outfall. Faunal composition is significantly distinct in this area.

Less impacted (LI): moderate declines in species richness and SDI, with abundance and biomass variable or enriched (bivalves and polychaetes); virtual elimination of echinoderms, crustaceans; elevated AVS and sterol indicators. Conditions suggest moderate or mild hypoxia in near-surface sediments. Faunal composition is significantly distinct from both the enriched zone and background zones.

Enriched (BE): no declines in species richness, abundance, juvenile recruitment or biomass or sediment hypoxia; enrichment of some taxa such as ophiuroids; depression of a few species; background AVS and 4-NP levels. This area does not show statistically significant differences in faunal composition from reference or far-field stations.

Background (R): the stations considered to be representing background or "reference" conditions. In the studies performed there are significant differences in faunal composition from the northernmost to the southernmost of these stations, which in our particular case is probably related to the S to N gradient of input to sediments from the south arm of the Fraser River. However, the faunal complement of the aforementioned stations is considered to encompass the natural range in biotic composition for this region and habitat. Faunal composition is significantly distinct from the impoverished zones (MI, LI), but not enriched zones (BE).

Confounded: within a proposed gradient, apparently anomalous results may occur. This can be due to any number of factors, including other anthropogenic inputs or activities such as bottom trawling (fishing). One of the stations identified in our studies shows erratic and patchy disturbance, with chemistry suggestive of periodic dumping of dredged material sandier than the other stations. No organic enrichment is suggested (high SDI), but faunal composition tends to be significantly distinct from other stations. Two other stations show faunal impoverishments in species richness, abundance and sometimes biomass, unrelated to organic enrichment (normal SDI and no enrichment opportunists). Near-surface species and those sensitive to surface disruption are reduced, suggesting physical disturbance variable in extent and magnitude, sometimes evident in a replicate sample for the surrounding stations.

Cautions, warnings and triggers are based on the 95th percentile of the cumulative frequency distribution for relevant indicators for a given zone over time. Since a variability of up to 20% in sampling precision is considered acceptable for benthic marine grab samples, a given sample must fall more than 20% outside the limit of the 95th percentile for the range to be considered a reliable and "real" change in the condition of the indicator in question.

Cautions provide early indication of changes in background reference conditions in the selected reference stations. Caution levels apply when any new reference samples fall outside plus or minus 20% of existing 95th percentile ranges for reference zones for a suite of relevant indicators.

Warnings apply for selected indicators which vary more than 20% from the 95th percentile range for any 3 replicate samples over a two year sampling period. The specific warning ranges vary for different pre-determined effect zones identified in the historical monitoring data; these zones include moderately impoverished, low impoverished or biotically enriched.

Trigger levels are not statistically derived, but based on best professional judgment and experience in other jurisdictions with similar types of organic enrichment effects. Trigger indicators and levels are designed to prompt a response prior to projected environmental degradation. Triggers for all effect zones are based on change in relevant indicators from historical conditions, past warning levels, to plus or minus 50% of reference ranges (based on the 95th percentile range for a given year) for any 3 replicate samples over 2 sampling years. All caution, warning and trigger levels must be reached concurrent with significant increases in AVS and 4-nonylphenol as chemical indicators of sewage derived inputs.

Higher Trophic Levels

Tissue residue objectives apply where provincial site-specific Water Quality Objectives (WQO) exist, and are generally defined for fish although the guidelines upon which they are based frequently state that they apply to human consumption of edible tissue from fish and/or shellfish. Where WQO do not exist, guideline values will be considered. Unequivocal linkages between tissue contaminant concentrations and specific discharges cannot easily, if at all, be established, especially not in the case of transient or migratory fish. Consequently, tissue residue values are allocated cautionary status due to their importance in establishing health at higher trophic levels.

Animals more suitable for immobilized study are available in the form of bivalves. Caged bivalves, in particular Mussels (*Mytilus edulis* or *M. trossulus*), have been employed to determine local impacts on the marine environment in the Pulp and Paper Environmental Effects Monitoring (EEM) Program. Where local circumstances permit, this may be an extremely useful substitute for the fish survey. From a Provincial Objective and Guideline perspective these animals substitute well for fish and their parity with fish is acknowledged in that the established parameters are often for "fish/shellfish", with identical concentrations for substances in question. Significant progress in the use of caged bivalves for determining and tracing effects is being made by researchers, and the NWRI/GVRD initiative for comparative trans-Canada studies (Development of Bio-indicators study), using physiologically more complex indicators is an important part of this expanding knowledge and is being reported on elsewhere at this conference.

Responses to Cautions, Warnings and Triggers

The consequence of reaching or exceeding caution, warning or trigger levels is that certain responses are defined as arising out of an exceedance. These responses are in keeping with the nature of the exceedance. Consequently, in moving from warnings to triggers we have a graduated set of responses. These are Caution Responses:

Caution Response 1: Identification of cause (e.g., sampling or processing error, increased organic loading, natural region-wide phenomena, outside existing or new effects, etc.);

Caution Response 2: If liquid waste discharge is source – risk assessment of temporal trend and determination of response need; and

Caution Response 3: Intensified sampling to confirm identification of cause and predict progression towards Water Quality Guideline.

Warning Responses:

Warning Response 1: Identification of cause (e.g., sampling or processing error, increased organic loading, natural region-wide phenomena, outside existing or new effects, etc.);

Warning Response 2: If liquid waste discharge is source – risk assessment of temporal trend and determination of best means to respond; and

Warning Response 3: Intensified sampling to confirm identification of cause and predict progression towards trigger status.

Trigger Responses:

Trigger response 1: Identification of cause (e.g., sampling or processing error, increased organic loading, natural region-wide phenomena, confounding effects, etc);

Trigger response 2: If liquid waste discharge is source, review mitigation options with the Environmental Monitoring Committee, sanction with the GVRD and present to the Province; and

Trigger response 3: Implement approved mitigation.

Special Programs and Studies

In addition to the regular monitoring programs, extraordinary studies are taking place either by or with other institutions on behalf of or jointly with the GVRD. Some of these studies are highlighting and/or quantifying new or different areas of concern and provide vital supportive information: (1) ambient monitoring program for the Fraser River (GVRD/MWLAP); (2) ambient monitoring program for southern Georgia Strait (GVRD/IOS); (3) PCB monitoring in harbour seals – Georgia Strait and Puget Sound (MWLAP); (4) programs to study endocrine disruptive chemicals, persistent organic pollutants and other micro-contaminants (various partners including DFO, EC, University of British Columbia and Simon Fraser University); and (5) development of Bio-indicators Program (joint GVRD/NWRI).

The described process has been developed within the context of the commitments outlined in the District's Liquid Waste Management Plan. It is intended to be a predictive and adaptive process which will allow the GVRD to respond proactively to issues of environmental quality. To ensure involvement of the relevant stakeholders, an Environmental Monitoring Committee was structured as part of the process in the Liquid Waste Management Plan. This science-based Committee reviews the various monitoring designs and initiatives and provides critique and recommendations. Through integration of the efforts of the GVRD and its member municipalities together with those within the federal and provincial governments, the universities and their associates, a process unique in Canada has been developing on the West Coast. This process is consistent with the GVRD's commitment to sustainability as per the Sustainable Region Initiative.

Reference

GVRD. 2004. Greater Vancouver Regional District cautions, warnings and triggers: a process for protection of the receiving environment. Greater Vancouver Regional District, Burnaby, BC.

Strategy for the categorization of polymeric substances on Canada's Domestic Substances List. A. Séné, D. Dubé and N. Davidson. Environment Canada, Environmental Protection Branch, Gatineau, QC.

The *Canadian Environmental Protection Act*, 1999 (CEPA 1999) requires the categorization of substances on the Domestic Substances List (DSL) according to the following criteria: (1) greatest potential for human exposure, (2) persistent (P) or bioaccumulative (B), and (3) inherently toxic (iT) to human or non-human organisms. The DSL contains 4,000 polymers that are subject to categorization. The information available to the government on these is Chemical Abstract Service Registry number, chemical name, monomers composition, United States Environmental Protection Agency flags on the *Toxic Substances Control Act* inventory and the industry use and quantity codes reported for the year 1986. As most polymers are designed to be stable substances, it is predicted that a large proportion will meet the criteria for persistence in at least one medium. For this reason, it is most efficient to use the iT parameter in an elimination process before looking at the P and B criteria. Environment Canada's approach to determine DSL polymers' iT for non-human organisms applies sorting of polymers according to the presence of particular functional groups and/or chemical categories for which a concern has been established for the environment. Particular reactive functional groups or electronic charges which are known to be degradable, to potentially impact toxicological properties or to be potentially of concern for biota that are present in the polymer structure is cause for investigation.

Ecotoxicity testing framework for petroleum hydrocarbon-contaminated site soils as part of a Tier 2 site-specific assessment. G.L. Stephenson and N.C. Feisthauer. Stantec Consulting Ltd., Guelph, ON.

The ecotoxicity data used for most site-specific ecological risk assessments originate from the scientific literature. Site assessors appear reluctant to generate site-specific toxicity data, despite the advantages. Conducting single-species toxicity tests using a test battery of methods and species can generate data that reduce the uncertainty associated with the use of literature toxicity values. Site-specific data reflect and integrate site-soil conditions and characteristics. We developed an assessment framework to address the risk associated with the soil contact exposure pathway. This exposure pathway is most critical for ecological receptors that live in or are closely associated with soil (plants, soil invertebrates, and burrowing mammals). The framework consists of a phased approach to testing. The first phase uses acute and chronic screening tests with plants and soil invertebrates exposed to undiluted soil samples from the site. The second phase of the testing framework also uses acute and chronic tests with these groups of test organisms but uses soil dilution procedures or a contamination gradient to either identify threshold effect concentrations (acute/chronic) or to identify remedial targets (chronic). Different approaches to site soil assessment are used for different situations. The framework will be presented and these approaches to soil assessment discussed as they relate to the assessment framework.

Deterministic and probabilistic risk assessments of pharmaceuticals and personal care products in wastewater treatment plants effluents based on the lowest toxicity values. K.D. Bergh and F.C.P. Law. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

More than 200 pharmaceuticals and personal care products (PPCPs) have been found in samples of WWTP effluent and receiving waters throughout the world. Discharge of this large number of biologically active compounds into the aquatic environment raises concerns that these may have potential impacts on aquatic life. The purposes of the presentation are: (1) to conduct a deterministic risk assessment (DRA) and a probabilistic risk assessment (PRA) on these PPCPs; and (2) to rank these PPCPs according to their potential impacts on the aquatic environment. The DRA was conducted by calculating the ratios of the environmental exposure concentrations (EECs) and the predicted no-observable adverse effects levels (PNOAELs) to give a hazard quotient value for each PPCP. EECs were based on the highest literature reported concentrations. Aquatic PNOAELs were derived from the lowest field, laboratory, and/or model-predicted toxicity reference values. Specific aquatic animal species and biological endpoints (lethality, growth, reproduction, etc.) were not considered in the risk assessments. PPCPs were ranked by their respective HQ value as follows: very high (HQ 100-1000), high (HQ 10-99), medium (HQ 1-9) and low (HQ 0-0.9). PPCPs with HQ values ranked as medium or higher were then evaluated for inclusion in the PRA. Only PPCPs that had sufficient EEC and toxicity data points to create cumulative distribution curves were included in the PRA study. For those PPCPs that met these criteria, all reported toxicity reference values (TRVs) were plotted to establish species sensitivity distributions (SSDs) and EEC distributions were superimposed on the SSD to give two cumulative distribution curves. The fraction of TRVs that overlap the EECs was then determined from the curves for each PPCP.

Of the 112 PPCPs assessed by DRA, 48 were ranked as medium or higher; these included: vasodilators, stimulants, stantin drugs, fragrances (personal care products), lipid regulators, hormones, beta-blockers, beta-agonists, antiphlogistics, antidiabetics, antibiotics, anti-anxiety, analgesics, and antiepileptics. PRA studies were conducted for the following 7 PPCPs: 17-alpha-ethynylestradiol (hormone - ovulation inhibitor), carbmazapine (antiepileptic), ciprofloxacin (fluoroquinolone - antibiotic), Musk xylene (fragrance - nitro musk), propanolol (beta-blocker – antihypertensive), sulfamethoxazole (sulfanomide - antibiotic), and tetracycline (tetracycline - antibiotic). Only 17-alpha-ethynylestradiol had TRVs that overlapped with the EECs. This ranking of PPCPs should support decisions on the selection of candidate substances for future assessments, where studies on ecologically relevant endpoints such as impairment of growth, development and reproduction should be used to assess ecotoxicological effects. Based on the results of the present studies, the priority for future assessment appears to be 17-alpha-ethynylestradiol.

The application of the Canadian Council of Ministers of the Environment (CCME) Water Quality Index (WQI) as a screening tool to evaluate the suitability of waters for aquaculture. R. Paterson, H. Khan, R. Crewe and A.A. Khan. Newfoundland and Labrador Department of Environment, St. John's, NL.

One of the first steps in selecting a site for aquaculture is the assessment of water quality. The paper outlines the use of the Canadian Council of Ministers of the Environment (CCME) Water Quality Index (WQI) as a screening tool to evaluate the suitability of waters for aquaculture. The CCME WQI was developed as a means to summarize large amounts of water quality data into simple terms (e.g., good poor, etc.) for reporting to management and the public in a consistent manner. It measures the scope, frequency and amplitude of water quality exceedences against guidelines. The paper outlines how the CCME WQI can be used to compare water quality against the CCME Guidelines for the protection of aquatic life and against other guidelines, that are specific to particular fish species. When used to evaluate and rank sites for aquaculture, the resultant rankings are consistent, easy to use when comparing sites and are easy to communicate to the public. The tool can also be automated to rank and screen a large number of prospective sites using water quality data stored in a database. To demonstrate the application of the CCME WQI a large number of fish specific guidelines were compiled from a review of available aquaculture literature.

Ecological risks due to current PAH loading from Sydney Tar Ponds to Sydney Harbour. D.W. Smith[1], W. Van Veen[2] and B. McRoberts[3]. [1]CRA, Inc., Exton, PA; [2]MGI, Inc., Sydney, NS; and

[3]MGI, Inc., Dartmouth, NS.

Muggah Creek, a tidal creek to Sydney Harbour, Nova Scotia is heavily contaminated with PAHs. As part of the remedial investigation for downstream portions of the Creek, appropriately called the Sydney Tar Ponds, we assessed ecological and human-health risks from current loading to Sydney Harbour. Using available data and mathematical modeling, risks to most sensitive receptors, human consumers of PAH-contaminated lobster and benthic macroinvertebrates in the Harbour, were estimated. No significant human health risks were identified, but assessment of ecological risks was uncertain. Magnitude of risks to benthic invertebrates varied greatly depending on three sources of uncertainty: (1) which sediment benchmarks (LEL, SEL, PEL, or site-specific benchmarks) best predict impacts; (2) whether current loading, as opposed to historical loading, was the primary cause of current concentrations; and (3) whether sediment reservoirs of PAHs in the Tar Ponds were subject to catastrophic resuspension. More conservative scenarios suggested significant risks from current loading from the Tar Ponds to the Harbour. Scenarios assuming steady-state sediment concentrations and PELs as valid benchmarks estimated impact to benthos in about 8 km2 of the Harbour sediments. Less conservative, potentially most likely scenarios suggested that current efflux of PAHs from the Tar Ponds posed little risk to benthic invertebrates in the Harbour. The magnitude of remediation also varied dramatically across risk scenarios, from essentially 0% to 100% of the Tar Ponds. These results illustrate a common problem facing risk managers, making very expensive decisions on very uncertain results.

A decision-making framework for contaminated sediments. P.M. Chapman[1] and J. Anderson[2]. [1]EVS Environment Consultants, North Vancouver, BC; and [2]Environment Canada, Environmental Conservation Branch, Burlington, ON.

Progress in addressing contaminated sediments has been slow due to difficulties in reaching consensus on the conduct of scientific assessments. A decision-making framework designed for the Canadian side of the Great Lakes but applicable elsewhere is described. In addition to an emphasis on common sense, this framework has four rules: (1) with only one potential exception, sediment chemistry data are not to be used alone for remediation decisions; (2) remediation decisions will be based primarily on biology; (3) lines of evidence (LOE) such as laboratory toxicity tests and models that contradict the results of field surveys are clearly incorrect; and (4) if the impacts of a remedial alternative will cause more environmental harm than good, then it should not be implemented. The framework is iterative and sequential in both scope and decision points. Sediments with contaminant concentrations below sediment quality guidelines (SQGs) that predict toxicity to less than 5% of sediment-dwelling infauna, and which contain no quantifiable concentrations of substances capable of biomagnifying, are excluded from further consideration, as are sediments that do not meet these criteria but whose contaminant concentrations are equal to or below background concentrations. Biomagnification potential is addressed by modeling; toxicity (acute and chronic) and alterations to resident communities are addressed by, respectively, laboratory studies and field observations. Individual decision points initially comprise relatively simple "yes" or "no" criteria. The ultimate decision point for sediments that cannot be so readily assessed, is a weight of evidence (WOE) matrix framework combining four main LOE: chemistry, toxicity, community alteration, and biomagnification potential.

Draft environmental screening assessment of polybrominated diphenyl ethers in Canada. J.P. Pasternak[1], L. Suffredine[1] and K. Taylor[2]. [1]Environment Canada, Environmental Protection Branch, Vancouver, BC; and [2]Environment Canada, Environmental Protection Branch, Gatineau, QC.

The *Canadian Environmental Protection Act* (CEPA 1999) requires the Ministers of the

Environment and Health to conduct screening assessments on substances that meet the categorization criteria specified in section 73. As part of this program, seven polybromodiphenyl ethers (PBDEs), used extensively as flame retardants, were selected to undergo a screening assessment. The seven PBDEs (those with 4–10 bromine atoms) evaluated in the screening assessment are present in three commercial mixtures, pentabromodiphenyl ether, octabromodiphenyl ether and decabromodiphenyl ether. Based on evidence relating to the substances' persistence, bioaccumulation potential, environmental concentrations, transformation and a risk quotient analysis, it is concluded that the subject PBDEs are entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity and are considered to be "toxic", as defined by CEPA 1999 Paragraph 64(a). The tetraBDEs, pentaBDEs, and hexaBDEs are persistent and bioaccumulative in accordance with the CEPA 1999 Regulations, their presence in the environment results primarily from human activity, and they are not naturally occurring radionuclides or naturally occurring inorganic substances. Notice therefore is further given that the Ministers of the Environment and of Health propose to implement virtual elimination under subsection 65(3) of tetra-, penta- and hexaBDEs. The draft assessment was released on May 8th, 2004, for a 60-day public comment period. Following consideration of the public comments, the report, including its CEPA toxic conclusions, will be revised as necessary.

Categorizing the Domestic Substances List: update on "Unknown or Variable composition, Complex reaction products and Biological materials" (UVCBs). S. Schnabel, A. Okonski, A. Séné and J. Gauthier. Environment Canada, Existing Substances Branch, Gatineau, QC.

The *Canadian Environmental Protection Act* 1999 requires the Minister of the Environment and the Minister of Health to categorize the Domestic Substances List (DSL) by September 2006 to determine which substances require further screening assessment. The DSL contains about 23,000 substances belonging to many different chemical classes. About 4,400 have been identified as UVCBs (Unknown or Variable composition, Complex reaction products and Biological materials), and most are data-poor. Environment Canada has developed a strategic approach for categorizing UVCBs. As a first step, UVCBs have been grouped into six major "blocks": organics, inorganics, organic metal salts, organometallics, polymers, and biologicals. Where possible, UVCBs in each "block" were further sub grouped based on chemical properties, moieties of concern and professional judgment. UVCBs will then be categorized according to general approaches developed for the corresponding substance class.

Assessing ecotoxicity studies submitted under the *New Substances Notification Regulations* in Canada: scoring method and regulatory uses. M.J. Lapointe[1], A. Pigeon[1], M. Lortie[1], M. Lewis[1], A.J. Atkinson[1], R.L. Breton[2], R. Thompson[2], G. Gilron[2]. [1]New Substances Branch, Environment Canada, Hull, QC; and [2]Cantox Environmental Inc., Ottawa, ON.

New substances intended to be imported into or manufactured in Canada must be notified to Environment Canada under the *New Substances Notification Regulations* (NSNR) of the *Canadian Environment Protection Act*. Upon receipt of these notifications, evaluation groups at Environment Canada and Health Canada conduct environmental and human health risk assessments based on information supplied by the notifier and other information available to the Departments.

The New Substances Program receives approximately 800-1,000 New Substance Notifications (NSN) per year. The total number of NSNs received now exceeds 13,000 since the inception of the program in 1994. Some of these notifications are accompanied with ecotoxicity studies; indeed to date the program has received more than 700 ecological toxicity studies. Although these experimental studies are not available to the public, they are useful to Environment Canada when assessing analogous new substances. This presentation describes a multifunctional electronic tool and database designed to: (1) provide a detailed electronic record of the studies, allowing access to key study parameters;(2) evaluate and compare the quality of ecotoxicity studies received by Environment Canada; (3) provide transparency in decision-making; (4) build Program consistency in evaluating the quality of studies; (5) provide ready access to surrogate data for the assessment of analogous substances; (6) provide summary reports for ecotoxicity study validation in assessment reports; and (7) once validated and scored, high ranking tests could be utilized within a QSAR model.

Development of a surface water quality objective for uranium for application in northern Saskatchewan. T.S.T. Moulding. Saskatchewan Environment, Environmental Protection Branch, Saskatoon, SK.

In order to ensure that the uranium industry in northern Saskatchewan is managed and developed in a sustainable manner, environmental protection must be ensured. To this end, environmental management tools, including effective and meaningful water quality objectives, are required. The development of a surface water quality objective (SWQO) for uranium for use in the management and development of U resources in northern Saskatchewan has therefore been undertaken. The process to develop this water quality objective will involve the preparation of a protocol, the compilation of information, and the derivation of the SWQO. This process will be used to determine the maximum concentration of U that will afford an acceptable level of environmental protection to aquatic ecosystems in northern Saskatchewan. Once determined, this concentration will be used as a SWQO for U for application in northern Saskatchewan. This will be accomplished by compiling U toxicity information, including the results from new toxicity studies that are being conducted specifically for this objective. Other factors including; (1) the relative importance of the study species to the various regional stakeholders; (2) relative importance of the species to northern Saskatchewan food webs; (3) the inherent differences between laboratory and field conditions; and (4) the level of uncertainty associated with the toxicity tests conducted will be used to determine the final SWQO value. An additional goal of this research will be to incorporate regionally relevant information while remaining consistent with the intent of the Canadian Council of Ministers of the Environment (CCME) protocol used for environmental quality guideline development in Canada.

Challenges in amphibian and reptile risk assessment and possible paths forward. L.J. Marshall, R.D. Willis and C.E. Moore. Cantox Environmental Inc., Halifax, NS.

Amphibians and reptiles may be particularly sensitive to environmental chemical stressors as a result of certain aspects of their ecology and physiology, such as the permeability of their skin, their limited home ranges and habitat preferences, position in the food web, life history, etc. However, these species have historically been excluded from evaluation in ecological risk assessments for a number of reasons including: (1) limited toxicity data; (2) lack of standardized laboratory toxicity test protocols; (3) lack of information on exposure parameters and life history; and (4) difficulties in estimating exposure, particularly via food ingestion. With increased awareness of the potential sensitivity of this receptor group, there is increased interest in including amphibians and reptiles in ecological risk assessments. As such, there is a need to identify data gaps and key resources that can be used to assess these species. This poster will outline the key challenges of including amphibians and reptiles in ecological risk assessments. In addition, it will provide a compilation of useful resources and information that facilitates their evaluation in an environmental risk assessment.

An update on Environment Canada's screening assessment activities for perfluoroalkyl compounds. A. Miettunen. Environment Canada, Exiting Substances Branch, Gatineau, QC.

Under the *Canadian Environmental Protection Act*, 1999 (CEPA 1999), the federal Ministers of the Environment and of Health must categorize the approximately 23,000 substances listed on Canada's Substances List (DSL). The initial categorization phase assists in setting priorities for assessment and involves identifying substances that may present to individuals in Canada the greatest potential for exposure; or, are persistent or bioaccumulative, and inherently toxic to humans or to non-human organisms. Priority for assessment is based on analysis of categorization results, as well as additional information from: industry, emerging science, international assessment activities, new substances notifications, public nominations, and provincial or international decisions. Environment Canada is currently assessing numerous chemicals on a pilot project for screening assessments as well as other prioritized substances, including certain perfluoroalkyl compounds. An update is presented on the screening assessments of: (1) perfluorooctane sulfonate (PFOS) and its precursors; and (2) perfluorooctanoic acid (PFOA) and its salts. A review is also given for the data gathering activities for other perfluorinated carboxylic acids (PFCAs), some of which have been identified as emerging contaminants of concern due to their persistence, high bioaccumulation potential and presence in the Arctic.

Amending environmental regulations to protect aquatic ecosystems from biological pollutants. A.J. Niimi. Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON

A sharp increase in the number of exotic aquatic species reported in different countries can be attributed to the production and transport of commerce goods and materials to global markets by transoceanic vessels. Regulatory agencies have recognized the need to develop the tools and authority to control or ecosystems which could be catastrophic once a species is established. Abrupt changes in biological community structure and function can be difficult to remediate. The North American Free Trade Agreement identified the need although the document is not legally binding but does recognize the need for proactive action particularly for species known to be invasive. The *Fisheries Act, Canada Water Act* and *U.S. Clean Water Act* includes provisions to protect these resources through conservation, prevent pollution and effective management, where chemical and physical agents are described as potential pollutants. These regulations have the framework to assess the risk of exotic species introductions, and scope to develop rapid control and eradication initiatives, but do not have the authority to act. The latter could be resolved by recognizing organisms as potential biological pollutant. Amendments to these and similar acts would provide regulatory agencies the authority to take an aggressive action, as the need arises, particularly with first reports of a new species in North America who invasive actions are well known.

Microbial source tracking – identifying non-point sources of fecal pollution. G.C. van Aggelen and H. Osachoff. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

The Environmental Toxicology section at the Pacific Environmental Science Centre (PESC) in North Vancouver, BC. has a Microbial Source Tracking (MST) technique that has been adapted and developed based on the published articles by Dr. Katharine Field from Oregon State University, Corvallis, OR. This MST technique is a genetic assay that detects genomic DNA from the host-specific intestinal bacterial group *Bacteroides-Prevotella* and thereby identifies the organisms responsible for fecal contamination in fresh or marine water samples. Currently at PESC we are able to distinguish between fecal contamination caused by humans, ruminant animals, and/or pigs. The procedure requires one litre of water taken from a potentially contaminated area and, after sample processing using molecular biology techniques, the results indicate presence/absence of fecal material from humans, ruminant animals, and/or pigs. A fecal coliform analysis is conducted in parallel because this MST procedure is not quantitative. The method is currently being expanded to work on shellstock and sediment samples.This technique has been used on marine and fresh water samples from around B.C. (2002-2004 projects) as well as sites in Newfoundland (summer 2004 projects). MST is a tool to aid First Nations, community groups, and all levels of government in identifying sources/organisms causing high fecal coliform counts in water systems.

A relative risk ranking of the National Pollutant Release Inventory (NPRI). A.M. Dunn. Environment Canada, Environmental Protection Branch, Dartmouth, NS.

The National Pollutant Release Inventory (NPRI) provides pollutant release and transfer data from point sources to various media in the Canadian environment. While the inventory serves as Canada's community right-to-know program, the growing number of listed substances and reporting facilities makes it exceedingly difficult for the public to discern which substances are of greatest concern in their respective communities. A chemical's impact is best characterized when its release data is combined with its toxicity and environmental fate properties. Presently, the NPRI does not provide a synthesis of this critical information and there is a need to provide more context for NPRI data to increase its usability. To help deliver this context and guide future internal priority setting, a relative risk ranking has been compiled for a subset of NPRI substances. According to the general paradigm of "Risk = Toxicity x Exposure", a ranking of NPRI substances was developed using a modified CHEMS model. Release-weighting factors were derived using NPRI data. Factors for each substance in each media were determined relative to the largest chemical release in each respective media. A risk score was then tabulated by multiplying the sum of release-weighted toxicity endpoints (i.e. acute/chronic human health and environmental toxicity) by the sum of weighted exposure factors (i.e. environmental half-life, log Bioconcentration Factor). While the model makes use of toxicity and exposure data, the risk ranking produced does not represent a risk assessment. Rather, the results from CHEMS should be viewed as a quantitative risk ranking used to prioritize substances for future risk management activities. Limitations included the reliance on modelled data for exposure parameters and the use of default values for data gaps. In spite of its limitations, the CHEMS risk ranking scheme provides a useful tool for prioritizing NPRI substances.

The relationship between sediment type and benthic macroinvertebrates mediated through aquatic macrophyte development: experimental sediment exchanges between natural and constructed wetlands on oil sands leases, near Fort McMurray, Alberta. L. Barr[1], N. Cooper[2], L. Foote[2], W. Tedder[3] and J.J.H. Ciborowski[1]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; [2]Department of Renewable Resources, University of Alberta, Edmonton, AB; and [3]Suncor Energy Inc., Fort McMurray, AB.

Post-mining land reclamation is a very important step in the oil sand mining region of Alberta's Athabasca deposit. Mining companies must restore the land to a capability equivalent to pre-mining conditions. We investigated the suitability of consolidated oil sands mine tailings (CT) as a sediment layering material in constructed wetlands for development of macrophyte and invertebrate communities. Plots were delineated in two reference wetlands and one experimental wetland (4-m depth of CT and oil sands process water (OSPW)). Reciprocal sediment transplantation provided two treatments in each reference wetland (control and CT) vs. three treatments in the CT wetland. This permitted us to distinguish effects of tailings sediment (CT vs. natural in each wetland) from those of oil sands process water (experimental vs. reference wetlands). To investigate colonization by invertebrates, we collected sediment cores and dip net sweeps from each sample location after 3, 12, 15, 24 and 27 months. Tailings sediment's lack of seed bank initially inhibited macrophyte presence in both reference and experimental wetlands. Midge larvae (Chironomidae) were the dominant invertebrates. In the initial study year, chironomid densities were significantly greater but overall taxa richness was lower in the CT wetland than the reference wetlands ($p<0.05$). Neither sediment type nor macrophyte abundance within sample locations influenced chironomid density. No significant differences were found in comparisons made 12 or more months after the start of the study. At the temporal scale of this study, inhibitory effects of oil sands tailings sediments on invertebrate colonization appear to be transient.

Toxicants in surface waters in two Nigerian coastal communities. G.R.E.E. Ana, M.K.C. Sridhar[1] and G.O. Emerole[2]. [1]Division of Environmental Health, University of Ibadan, Ibadan, Nigeria; and [2]Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

Nigeria's fragile coastal ecosystems is characterized by considerable industrial activities which discharge wastes that increase the burden of PAH compounds and other toxic elements in the environment but regrettably information is scanty. The objective of this study was to determine the levels of some inorganic elements especially heavy metals including Pb and organic compounds particularly PAH in surface waters from two selected communities of the Niger Delta Area viz Eleme (exposed community) with refineries and petrochemicals and Ahoada East (control community) without such industries. An experimental study design was used. A total of 18 surface water samples (9 from Eleme and 9 from Ahoada East) from rivers, streams and creeks were collected systematically into 1 L glass and plastic containers. Samples were analyzed using standard methods for pH value, inorganics viz SO_4, PO_4, Zn, Fe, Ni, Cd and Pb. Samples were also processed according to standard methods and analyzed for organics viz Phenol and for PAH compounds using high performance liquid chromatography with fluorescence and UV detectors. Results indicated that the mean pH value at Eleme was 5.72±0.75 as compared to 4.79±1.17 at Ahoada East. Heavy metals particularly Pb recorded higher mean value(0.75±0.06 mg/l) at Eleme as compared to Ahoada East(0.15±0.11 mg/l) though values were lower than FEPA limits. The total PAH concentrations were higher in surface waters from Onne stream close to the Sea Port (8.89×10^4 ng/l) and from Okirika creek contiguous to Alesa, which receives the refinery effluents (3.58×10^4 ng/l). The highest concentration of Benzo (a) pyrene the main toxic indicator was recorded in the streams at Ogale (2.01×10^4 ng/l) and Eteo (1.98×10^4) all at Eleme.The mean total PAH levels in surface waters at Eleme ($2.21\times10^4\pm2.76\times10^4$ ng/l) as compared to Ahoada East ($8.39 \times 10^3\pm1.46\times10^4$ng/l) were 3 folds higher and also higher than the WHO guideline limits of 50 ng/l. This study reveals that surface waters from Eleme communities where there are more industries are more polluted with toxicants such as PAH and heavy metals like Pb than are those from Ahoada East. The implication is that populations residing in these communities may be more vulnerable to health risks associated with exposure to these compounds. There is therefore need to adopt cleaner technologies by industries in this region.

Sediment Disposal at Sea / Immersion en mer des sédiments

Session co-chairs: R.C.H. Wilson and P.A. Topping

Current research and monitoring of Environment Canada's disposal at sea program. P.A. Topping. Environment Canada, Environmental Protection Service, Gatineau, QC.

Environment Canada controls disposal at sea activities by a permit system under the Canadian *Environmental Protection Act*, 1999 and related regulations. Each year in Canada, two to three million tonnes of material are disposed of at sea. Most of this is dredged material that must be moved to keep shipping channels and harbours clear for navigation and commerce. As well, every year, Environment Canada conducts long-term monitoring at representative disposal sites to verify permit decisions and respond to Canada's national and international reporting obligations. The Department also conducts research to examine questions related to managing sediments and provide tools to aid permit assessments. This paper will examine some recent questions being considered by Environment Canada concerning the development of toxicity tests and the management of sediments at disposal at sea sites.

Integrated techniques for monitoring of an offshore disposal site in a dynamic tidal environment. R.D. Parrott[1], M.B. Parsons[1], M.Z. Li[1], V.E. Kostylev[1], J.E.H. Clarke[2], A. Duxfield[2] and K.-L. Tay[3]. [1]Natural Resources Canada, Geological Survey of Canada, Dartmouth, NS; [2]Department of Geodesy and Geomatics, University of New Brunswick, Fredericton, NB; and [3]Environment Canada, Environmental Protection Branch, Dartmouth, NS.

Material dredged from shipping channels and around the wharves in Saint John Harbour, NB, has been placed in an exposed site in the harbour approaches in an area affected by strong currents, 8 metre high tides, and waves from winter storms. In 1999, a joint project was initiated between Environment Canada and the Geological Survey of Canada to define the zone of influence of the dumping; assess the physical, chemical and biological impacts caused by disposal activities; and evaluate the long-term suitability of the site. The study utilized integrated geophysical, sedimentological, geochemical, and biological techniques to characterize the nature, distribution and remobilization of marine sediments. Repetitive multibeam bathymetry surveys showed accumulation of dredged spoils due to disposal in the summer, and transport and redistribution by currents and waves over the winter months. Seafloor samples, photographs, and video transects were analyzed to asses the physical and chemical characteristics of the seabed habitats and composition of benthic communities. Current meter results were used to develop a 2-dimensional hydrodynamic tidal model and a sediment transport model. The models show that fine sand could be mobile throughout 54% of the tidal cycle, and that transport rates during a one-year storm could be 1-2 orders of magnitude greater than from the tidal current. Geochemical results show that the sediments are relatively uncontaminated. Trace metals detected in the sediments were used to trace the dispersion of dredge spoils. The integration of the various data sources provided a more comprehensive interpretation of the nature of the disposal site than any of the techniques alone could have provided.

Environmental monitoring of dredged material ocean disposal sites in Atlantic region. K.-L. Tay[1], K.G. Doe[2], A. MacDonald[1], R. Mroz[1], K. Lee[3] and R.D. Parrott[4]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [2]Environment Canada,; Environmental Science Centre, Moncton, NB; [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [4]Natural Resources Canada, Geological Survey of Canada, Dartmouth, NS.

Environmental monitoring of dredged material ocean disposal sites requires special equipment and technologies. This paper describes the use of integrated geophysical, geochemical, and biological techniques for this type of monitoring. The zone of physical impacts (spatial extent) at the disposal sites is measured using acoustic techniques (sidescan sonar, multibeam, and sub-bottom profiler surveys). The temporal changes, erosion and stability of the disposed materials are measured by repetitive multibeam surveys and the deployment of the benthic annular flume and current meters. Based on the spatial and temporal information obtained by the geophysical equipment, sediment samples are collected from the impacted areas to assess chemical contamination and potential biological effects. Geochemical data, bottom photographs and video are used to ground truth the information collected by the geophysical equipment. Data collected by the geophysical, geochemical, and bottom photographs are also used for benthic mapping and tracking of fisheries resources. Biological monitoring includes measurement of lethal (amphipod) and sublethal toxicities (Microtox®, sea urchin and polychaete) and benthic biota (abundance, number of species, diversity, evenness, and Bray-Curtis index). Data collected by the biological assessment are used together with the geophysical and chemical information to identify potential impacts of the disposed dredged materials on existing resources based on a weight-of-evidence approach. Case studies are used to demonstrate the effectiveness of these underwater monitoring techniques.

A survey of tributyltin residues at selected ocean disposal sites and harbours in Canada. J.A. Thompson[1], P.A. Topping[2] and R.C.H. Wilson[1]. [1]2WE Associates Consulting Ltd., Victoria, BC; and [2]Environment Canada, Environmental Protection Service, Gatineau, QC.

During the fall of 2003, a survey of five active Canadian coastal ocean disposal sites was undertaken by Environment Canada to determine concentrations of tributyltin (TBT) and its decomposition products dibutyltin (DBT) and monobutyltin (MBT) in sediments and interstitial waters removed from the same samples. The selected sites are located in the Bay of Fundy (Meteghan, NS; Black Point, NB), Gulf of St. Lawrence, (Cap-aux-Meules in the Magdalen Islands, QC), and the Strait of Georgia (Sand Heads and Point Grey, BC). In addition, samples of sediments from potential load sites were collected at Vancouver, Victoria and Esquimalt harbours, BC, and at Cap-aux-Meules Harbour, QC. The aim of the survey was to establish the concentrations of this toxic marine antifoulant in sediment and pore water, and to assess them against current sediment quality guidelines. Pore waters were separated from the sediment matrix by centrifugation under a nitrogen atmosphere. Samples were treated using derivatization/solvent extraction followed by analysis with gas chromatography/atomic emission detection (GC/AED). Most of the samples from the disposal sites were found to be below the limit of detection. In some samples from three of the disposal sites, TBT concentrations were found in bulk sediments from 1.0-12 μg/kg. Concentrations of TBT in bulk sediments ranging from 1.0-920 μg/kg were found at all of the harbour locations (located near shipyards or ferry terminals). Butyltin compounds in pore water from harbour sediments ranged from the limit of detection to 6.1 μg/L. Of note were the quantities of the degradation products (DBT and MBT) found in harbour samples from Esquimalt and Victoria. Statistical analyses of the data and the significance of some high quantities of TBT for the viability of the benthos will be discussed. This is among the first reports of TBT in sediment pore water in Canada.

Sediment chemistry and toxicity at the Point Grey disposal site. R.C.H. Wilson[1], S. McKinnon[1] and D. Sullivan[2]. [1]2we Associates Consulting, Victoria, BC; and [2]Environment Canada, Environmental Protection Branch, Vancouver, BC.

The site for disposal at sea off Point Grey, British Columbia, is the most intensively used on Canada's west coast. This presentation focuses on the chemistry and toxicity of sediment samples collected by Environment Canada's site monitoring program between 1975-2001. Sediment trace metals data, with samples sizes exceeding 600 over the 26-year period, were not log-normally distributed; other transformations generally failed to produce normal distributions. Spatial and temporal analysis shows that sediment chemistry patterns are related to operation of the disposal site and to the influence of natural sedimentation. Concentrations of most trace metals within the disposal site boundary have been decreasing over time, while at the same time the particle size spectrum has been getting coarser. PAH concentrations have been increasing over time at similar rates within and outside the disposal site boundary, suggesting that disposal activities are only partially responsible for this temporal trend within the site. Averaged concentrations of regulated parameters lie between the *Interim Sediment Quality Guidelines* (BC and CCME) and the Probable Effects Levels. Bioassays using two species of amphipod, a sea urchin, and the Microtox® solid phase protocol do not show consistent results. Correlations between sediment chemistry and sediment toxicity vary over the range of tests used, both at Point Grey and at other disposal sites on the West coast.

Sand and surf – a new approach to managing Fraser River dredge spoils. S. Standing[1] and P. Hill[2]. [1]Environment Canada, Environmental Protection Branch, Vancouver, BC; and [2]Natural Resources Canada, Geological Survey of Canada, Sidney, BC.

Analysis of sediment transport trends, particle size distribution, and known geophysical and oceanographic processes on Roberts Bank and Sturgeon Bank suggest that the area is experiencing considerable change. As well, the physical implications of climate change are likely to be significant, including substantial shrinking of the tidal flat area. While the reasons for the changes are not clear, increased river channelization, anthropogenic influences, natural sea level rise, the development of the Delta Port Terminal, and highly variable wave action and sea conditions are likely contributing factors. Consequently, Environment Canada launched a two year study to assess Fraser River sand disposal management options. This research may lead to innovative management options for regulators and industry. Regulated by Environment Canada under the provisions of the *Canadian Environmental Protection Act*, the Sand Heads ocean disposal site received enough sand in the last two years to fill BC Place Stadium. The objective of this study is to evaluate and compare the geological, oceanographic and biological implications of the current disposal site and management practices and, if appropriate, identify placement options that would benefit the Roberts Bank ecosystem. If suitable placement options are identified, deemed appropriate, as well as logistically and economically manageable, alternate resource management options will need to be considered. Changes to existing resource management structures may influence a broad range of evolving community, industrial and conservation interests throughout the lower Fraser.

Pacific and Yukon Region disposal at sea website. J.E. Wilkinson. Environment Canada, Environmental Protection Branch, Vancouver, BC.

The Disposal at Sea website is an integral part of communication between Environment Canada and other government agencies, industry, non profit organizations and members of the general public. General information including what, why, where, when and who is involved in disposal at sea is readily available to all viewers. Photographs and videos are also provided to enhance the viewer's understanding and perception of the Disposal at Sea Program. The website also offers specific information about the Disposal at Sea permitting process and guides the viewer through the permit application process. All of the Disposal at Sea program guidelines, standards and publications are also easily accessible through the website.

Disposal at sea marine sediment guideline for cadmium: review and recommendations for the British Columbia coast. C.D. Campbell, R. Leung and D.L. Sullivan. Environment Canada, Environmental Protection Service, Vancouver, BC.

Material approved for ocean disposal under the *Canadian Environmental Protection Act*, 1999 (CEPA) must meet the criteria in the *Disposal at Sea Regulations*, 2001 and the *Interim Contaminant Testing Guidelines* (ICTG). The finalization of the guideline for cadmium (0.6 mg/kg dry weight) is a priority issue for Environment Canada's Disposal at Sea Program. Marine sediment samples collected during the three sampling events spanning 1987-2001 (n=299) were examined with regards to risks, if any, posed to environmental and human health. Statistical analysis showed Cd concentrations in sediment samples were not significantly different than the ICTG. Geographically, no pattern in exceedance of the ICTG was apparent. Preliminary bioassay testing did not support the concept that toxicity was related to increased concentrations of Cd. Numerous sediment samples contained Cd concentrations below the regulated limit and ICTG, yet elicited toxic responses in amphipod bioassays. When Cd concentrations substantially exceeded established values, Cd bioavailability was not a concern, and adverse impacts did not always occur. Results show many inlets on the British Columbia coast naturally contain Cd concentrations higher than the regulated limit and ICTG; therefore, continued study and review of the screening limit currently used by Environment Canada's Disposal at Sea Program is warranted. A more appropriate guideline level could be developed to take into account natural Cd levels off the British Columbia coast.

Wood waste associated with dredged materials: potential management options. R. Leung and S. Standing. Environment Canada, Environmental Protection Branch, Vancouver, BC.

Environment Canada initiated a study using an eco-industrial networking approach to examine the feasibility of using wood waste associated with dredging and subsequent disposal at sea in alternative waste management options. Specifically, the study determined whether disposal at sea was the most economically and ecologically sound management option for wood waste associated with dredged materials. In 2003, a quarter of 660,000 cubic metres of dredged materials from British Columbia's coastal waterways contained wood waste. Increasingly, wood waste is targeted as a potential resource for energy generation, value-added manufacturing or other processing. Combustion, biological conversion, pyrolysis, and substitution of wood waste were evaluated and were considered unfeasible due to logistical, economical and ecological constraints at this time. Disposal at sea remains an acceptable management option for dredged materials containing wood waste. A wood waste management initiative could be developed to control wood waste at log handling facilities.

Sand Heads ocean disposal site: managing risk. S. Standing and R. Leung. Environment Canada, Environmental Protection Branch, Vancouver, BC.

Environment Canada and Natural Resources Canada are working together to analyze the sediment regime in the area surrounding Sand Heads. This study assesses the geological and oceanographic implications of the existing disposal practices at the Sand Heads ocean disposal site. Since being designated an approved disposal site in 1974, Sand Heads has received over 12 million cubic meters of sand and silt, a by-product of on-going channel maintenance in the lower Fraser River. Located in an area of strong bottom currents and active submarine mass movements the material deposited at Sand Heads is likely to remobilize. While preliminary results indicate that ocean disposal activities on the south eastern end of the designated site may increase the risk of a slide on the delta's slope, existing management practices appear to be appropriate. Disposal at sea management practices may be amended to reflect the slope stability at Sand Heads.

Wood waste management initiative. J.E. Wilkinson and S. Standing. Environment Canada, Environmental Protection Branch, Vancouver, BC.

Wood waste is increasingly recognized as a misplaced resource and is targeted by many private and public sector interests as a potential resource for energy generation or other processing. In 2003, Environment Canada funded a study entitled Alternative Management Strategies for Dredged Materials Containing Wood Waste to assess the viability of several energy generating and value added processing management options for wood waste in dredged material (Unpublished Environment Canada Report, under review, 2004). Due to the high moisture and silt content of the wood waste associated with dredged materials, the results of this study suggest that disposal at sea remains the most appropriate management option. Based on these findings and Environment Canada's mandate to promote sustainability and pollution prevention, Environment Canada will promote wood waste management strategies to reduce the volume of wood waste disposed of at sea. The results of this study will compliment the Fisheries and Oceans Canada 2003 Guidebook: Environmentally Sustainable Log Handling Facilities in British Columbia (G3 Consulting Ltd., 2003) recommendations for wood waste control at log handling facilities, while addressing Environment Canada's waste management objectives.

Oil and Gas / Huiles et graisses

Session co-chairs: K. Lee and D. Taylor

Terra Nova Environmental Effects Monitoring program: from environmental impact statement onward. E. DeBlois[1], M.D. Paine[2], F. Power[3] and U. Williams[3]. [1]Jacques Whitford Environment Ltd., St. John's, NL; [2]Paine, Ledge & Associates, North Vancouver, BC; and [3]Petro-Canada, St. John's, NL.

In 1996, Petro-Canada submitted an Environmental Impact Statement (EIS) for the Terra Nova oil field development outlining anticipated project effects. As a condition of project approval, an Environmental Effects Monitoring (EEM) program was designed with input from stakeholders and international and national specialists. The primary purpose of the EEM was to test effects predictions made in the EIS. As such, the program was established to detect change in the quality of the receiving environment through examination of sediment quality, water quality and ultimately biological resources. Sediment toxicity, hydrocarbon content, metals concentration and benthic infaunal community structure were assessed as part of the program. Hydrocarbon, metal and chlorophyll concentrations were measured on water samples. Metals and hydrocarbon body burden, taste and morphometrics and life history characteristics were assessed for Atlantic scallop and American plaice. Mixed function oxidase induction assessment, haematology and histopathology were performed on American plaice tissue samples. The strength of the Terra Nova EEM program results from these many supporting program elements. A weight of evidence approach was used to assess project effects to the receiving environment. Since its inception in 2000, the program has proven effective at detecting very small inter-annual changes in the marine environment unrelated to project activity and therefore provides a powerful tool for detecting and identifying project effects outlined in the EIS.

Hibernia Environmental Effects Monitoring programs: sediment chemistry profiles and their implications for environmental impacts. S.A.Whiteway[1] and R.A. Dunphy[2]. [1]Jacques Whitford Environment Ltd. St. John's, NL; and [2]ExxonMobil, St. John's, NL.

The Hibernia field was discovered in late 1979. First oil occurred on November 17, 1997 and drilling commenced in June 1997. Hibernia will complete the drilling of at least 64 wells with the possibility of 83 development wells being required during the life of the project. On average Hibernia has used water based muds for the first 300-400 meters of well depths, with the remainder of the wells being drilled using a synthetic-based mud (SBM) system due to challenges posed in extended reach and directional drilling. Condition 12 of the project approval decision (Decision 86.01, CNOPB 1986) required that Hibernia conduct Environmental Effects Monitoring (EEM) programs to monitor the environmental impacts of this facility on the receiving environment. Hibernia will conduct its fifth post-production EEM this summer. During the first seven years of operation, Hibernia has made several operational changes resulted in improved environmental performance at the Hibernia Platform. The changes observed in the sediment quality surrounding Hibernia is an excellent case study that provides insights on the impacts of SBM discharges on the receiving environment, as well as possible implications associated with different drilling scenarios. (i.e multiple development/production wells versus single exploratory wells). This presentation will focus on the sediment chemistry profiles, specifically with reference to TPH and barium.

Hibernia Environmental Effects Monitoring programs: sediment toxicity data 1994-2004. S. Whiteway[1] and R. Dunphy[2]. [1]Jacques Whitford Environment Ltd., St. John's, NL; and [2]ExxonMobil, St. John's, NL.

The Hibernia Environmental Effects Monitoring (EEM) program employs a suite of toxicity tests to monitor the environmental impacts to the sediments surrounding the Hibernia Platform. The toxicity tests used at Hibernia are the Microtox® bioassay as a screening tool, with amphipods and juvenile polychaetes bioassays used for a selected set of stations. Amphipods and juvenile polychaetes bioassays have been used in the near field stations, controls and any stations for which a failure of the Microtox bioassays had been observed. The criteria established in 1995 for what constitutes a Microtox failure was developed in a similar manner as the new interim guidelines for assessing Microtox toxicity as outlined in the standard method (Environment Canada EPS 1/RM/42 2002). The toxicity tests used as a suite have been a useful tool for assisting in assessing the overall sediment quality at the Hibernia site. However, the examination of the responses for each type of toxicity tests has yielded interesting information. This presentation will focus on the sediment toxicity data since 1994.

Effective monitoring programs for oil development: the program for the Grand Banks of Newfoundland. J.F. Payne. Department of Fisheries and Oceans, Science Branch, St.John's, NL.

Although the majority of information available from field and laboratory studies (e.g. PERD) suggest that offshore impacts will likely be minimal, monitoring programs are required to assess hypotheses about the nature and scale of impacts as well as to possibly uncover new insights which can only come from field studies. The Canada Newfoundland Offshore Petroleum Board is the regulatory authority for development on the Grand Banks and the monitoring programs developed by industry have been formulated in a fairly integrated manner with guidance from regulators, nationally and internationally recognized experts and the fishing industry. Provision for input by the public through special fora has also been a key element in program development. In addition to chemical monitoring, the biological effects component in place is generally in line with concepts adopted by parties to the Oslo and Paris Conventions for evaluating the effects of chemicals in the marine environment. This includes sediment bioassays, benthic community responses and early warning measures of fish health such as induction of mixed-function oxygenases, and histopathology. Given the importance of the fishing industry, a special focus has also been placed on tainting and contamination studies on representative commercial species such as snowcrab, scallop and American plaice. Altogether, the monitoring programs in place on the Grand Banks should provide early warning of any potential problems related to sediment and water quality, fish quality and fish health. It is generally recognized that modified approaches may be required for different sites due to differences in type and magnitude of discharges and resources in an area. Also since EEM should follow a feedback process, caution is warranted with respect to locking into overly prescriptive or "harmonized" programs in a development area.

Effect of salinity on the uptake and toxicity of PAH from chemically dispersed oil. S.D. Ramachandran[1], M. Boudreau[2], S.C. Courteney[2], T. King[3], J.A. Dixon[3], M.J. Sweezey[1], and P.V. Hodson[1]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Chemical dispersants increase the toxicity of crude oil to aquatic species by increasing the miscibility and bioavailability of its components. Studies of CYP1A induction in freshwater rainbow trout have demonstrated that chemical dispersion increases exposure to polycyclic aromatic hydrocarbons (PAHs) by increasing the concentrations of suspended oil droplets and dissolved hydrocarbons. CYP1A induction increased by 6-1000 times in chemically dispersed crude oil as compared to undispersed oil. Because chemical dispersants are formulated for salt water, we compared PAH uptake indicated by CYP1A induction and toxicity of dispersed oil between fresh and salt water. Experiments with mummichogs at 15 and 30‰ measured the effects of increased salinity, while comparisons between rainbow trout and mummichogs at 15 ‰ controlled for species differences. Preliminary toxicity data show increased hydrocarbon concentrations in dispersed treatments with blue sac disease signs in freshwater medaka and brackish-water mummichog embryos exposed to higher concentrations of dispersed crude oil.

Toxicity of crude oil to early life stages of two fish species. L.M. Clarke[1], R.S. Brown[2], T. King[3], K. Lee[3] and P.V. Hodson[1]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Department of Chemistry, Queen's University, Kingston, ON; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

The early life stages of fish exhibit signs of dioxin-like toxicity when exposed to crude oil. Toxicity is correlated to the presence and concentration of alkyl-substituted polycyclic aromatic hydrocarbons (PAHs), and characterized by the presence and severity of blue-sac disease (BSD), a syndrome that includes edema, hemorrhaging, deformities, and induction of cytochrome P4501A (CYP1A) enzymes. We compared the extent of CYP1A induction and BSD in the early life stages of rainbow trout (*Oncorhyncus mykiss*) and Japanese medaka (*Oryzias latipes*) after exposure to two crude oils: Scotian Shelf (SS) and Alaska North Slope Crude (ANSC). Embryos were exposed to a concentration series of chemically enhanced water accommodated fractions of each of the oils. Each oil was unique in PAH composition and chemical characteristics, and we hypothesized that ANSC oil would be more toxic, as it had the greater PAH concentration. The prevalence and severity of BSD was characterized at swim-up, and the extent of PAH exposure was estimated by the extent of immunohistochemical staining of CYP1A protein. Preliminary data suggests that PAH exposure was greater for ANSC oil, consistent with a higher concentration of PAH, and that there the responses of the two species were strongly correlated.

Assessment of freshwater sediment toxicity from enhanced anaerobic vegetable oil degradation. K. Lee[1], G. Wohlgeschaffen[1], T. King[1], S.E. Cobanli[1], K.G. Doe[2], P.M. Jackman[2], B.A. Wrenn[3], Z. Li[3] and A. Venosa[4]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [2]Environment Canada, Environmental Conservation Branch, Moncton, NB; [3]Department of Civil Engineering, Washington University, St. Louis, MO; and [4]U.S. Environmental Protection Agency, Cincinnati, OH.

A proposed vegetable oil spill countermeasure for use in aquatic environments is based on the addition of clay to mediate the transport of oil to the sediment where it is degraded by anaerobic microorganisms. However, there is concern that components within the oil such as oleic acid and metabolic by products from enhanced biodegradation such as free fatty acids from the hydrolysis of vegetable oil triglycerides maybe toxic. To address this issue, the biodegradation and toxicity of canola oil within anerobic sediments in replicate test chambers (0, 17, 35 g oil/kg clay-sediment mixture) was monitored with the Microtox® and amphipod (*Hyalella azteca* - growth and survival) bioassays.

Gravimetric analysis of the residual oil and measurements of methane production confirmed that anaerobic degradation occurred even at the highest test concentration. The Microtox® Solid-Phase Test showed a trend towards the depression of EC50 values with increased oil concentrations at T=0.

After 2 weeks, both the oiled treatments showed elevated toxicity levels. However, by 2 months, the sediments of both treatments had recovered to control levels. A similar trend was observed for the results of the amphipod assay based on the monitoring of survival and growth parameters. These results suggest that concerns over the production of free fatty acids and other toxic metabolites from anaerobic hydrolysis of vegetable oil triglycerides in the proposed spill countermeasure strategy are not warranted.

Identification of CYP1A inducing compounds in crude oil. C.W. Khan[1], B.P. Hollebone[2], Z. Wang[2], R.S. Brown[3] and P.V. Hodson[1]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Environment Canada, Environmental Technology Centre, Ottawa, ON; and [3]Department of Chemistry, Queen's University, Kingston, ON.

Crude oil is a major source of polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment. PAHs are known to cause developmental malformations in the early life stages of fish and we have observed similar effects in larval fish exposed to crude oil in the laboratory. CYP1A induction is characteristic of developmental toxicity caused by crude oil, and is an effective biomarker of PAH uptake. Due to the complex chemical make-up of crude oil, it is uncertain as to which PAHs (or other constituents) cause toxicity. We used a Toxicity Identification and Evaluation (TIE) approach with different crude oils to separate bioavailable PAHs into sub-fractions, as indicated by the extent of CYP1A induction in rainbow trout after 48-h exposures to each fraction. Fractions were created by low temperature vacuum distillation, and corresponded to white gas (F1), kerosene (F2), coal tar/bitumen (F3), and wax (F4). Whole oil and some fractions induced hepatic CYP1A activity with a well-defined exposure concentration. F3, the highest in PAHs, accounted for most CYP1A induction in whole oil. F4 also caused moderate CYP1A induction, while F1 caused none. Comparisons of CYP1A induction potency with chemical analyses of fractions support the hypothesis that alkyl PAHs may be the most important source of CYP1A inducers in F3, while benzo[*a*]pyrene accounts for most of the CYP1A induction caused by F4.

Isolation of CYP1A inducing components in coal tar fraction (F3) of Alaska north slope crude oil – a preliminary study. G. Saravanabhavan[1], C.W. Khan[2], R.S. Brown[1] and P.V. Hodson[2]. [1]Department of Chemistry, Queen's University, Kingston, ON; and [2]Department of Biology, Queen's University, Kingston, ON.

There has been an increased concern about the effects of weathered crude oil on the early life stage of aquatic organisms, particularly fish. It has been reported that the exposure of larval fish to crude oil can lead to blue sac disease (BSD). Current experimental evidence suggests a relationship between the induction of CYP1A enzymes and the occurrence of BSD in fish species, though the mechanism of BSD is still unclear. Our primary goal is to contribute to the Toxicity Identification and Evaluation (TIE) approach by isolating CYP1A. In this process, we also include the development of an improved separation and analysis scheme for crude oil characterization. Earlier work in our group showed that the coal tar fraction (F3) of the crude oil, rich in polycyclic aromatic hydrocarbons (PAHs), gave the highest CYP1A activity. In addition to PAHs, the coal tar fraction contains several classes of compounds such as waxes, asphaltenes and resins. Therefore, the TIE should include initial separation of these compound classes followed by a detailed characterization of the PAH classes. We developed of a solvent extraction method to provide fractionation of F3 into compound classes, with the emphasis on the isolation of PAH components. Fractions rich in PAHs caused a marked CYP1A induction in juvenile trout (*Oncorhynchus mykiss*) while fractions poor in PAH did not. In comparison with solid phase extraction methodology, the solvent extraction procedure provided better PAH fractions for further analysis by liquid chromatography. The use of liquid chromatographic methods for further fractionation of the PAHs in the TIE study will be discussed.

Use of polyethylene membrane devices for monitoring diesel oil contamination on and within beaches. J.W. Short, S.D. Rice, M.R. Lindeberg and M.G. Carls. Auke Bay Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, Juneau, AK.

A small diesel fuel spill associated with commercial fishing activity was detected in Port Chalmers, inside Prince William Sound, Alaska, while the site was being monitored for other purposes. We had placed an array of 12 polyethylene membrane devices (PEMDs) on a beach; six on the beach surface and six buried about 0.5 m below the surface. The sampling devices were placed at 3 tidal elevations along 2 transects oriented perpendicular to the shoreline for 1 month during March 2002, and again during June. A small diesel oil spill occurred before June 23, 2002, associated with a fishery nearby, and was clearly evident in the chemical analysis of the PEMDs for polycyclic aromatic hydrocarbons (PAH), which increased several thousand-fold from a few hundred ng per device in the winter deployment. We also measured PAH in sediments and in interstitial water in the beach after the diesel spill. Comparison of these analyses showed the PEMDs provide a very sensitive and inexpensive method for monitoring interstitial water flow within these porous beaches.

Introduction

Movement of interstitial water within beaches fouled by oil spills has received little attention, but may be an important means of exposing intertidal biota to toxic components leached from the stranded oil (Carls *et al.* 2003). Oil stranded on and within beaches may be surprisingly persistent (Reddy *et al.* 2002, Short *et al.* 2004). When pools of oil are stranded on beaches composed of porous substrates, the oil may readily seep into the beach interstices as the water table follows the falling tide, and then become trapped by capillary forces among sediment contact points. Such trapped oil may resist flotation on subsequent tidal cycles for very long periods (decades). Tidally-driven interstitial water movement may be forced through interstices of oil-contaminated beaches, accumulating toxic polycyclic aromatic hydrocarbons (PAH) dissolved from the oil, and then transported considerable distances in unexpected directions, including to beach elevations above the sediments contaminated (Carls *et al.* 2003).

Monitoring the movements of PAH-contaminated interstitial water within beaches is difficult because of the low concentrations usually encountered, and because of flow patterns that are likely to be intermittent and often unpredictable. Discrete water samples may fail to include contaminated water flowing by the sampled point at times other than when samples are collected, requiring an intensity of sampling effort that in most cases is likely to be prohibitive. Integrative samplers, such as semi-permeable membrane devices (SPMDs) or the even simpler polyethylene membrane devices (PEMDs) efficiently accumulate organic pollutants from intermittent exposures, and hence are especially suited for such monitoring situations. Semi-permeable membrane devices consist of a polyethylene membrane enclosing a fluid lipid (typically triolein), which acts as a reservoir for the accumulated organics; the accumulated organics are retrieved by dialysis on retrieval from the field. The fluid filled SPMD samplers have the advantage that they can be constructed with suites of reference compounds (e.g. perdeuterated PAH) in the lipid phase, and measurement of the losses of these compounds provides an indication of the effective volume sampled by the device. The simpler PEMDs consist of only the polyethylene membrane of the SPMD, into which reference compounds cannot be easily incorporated, but easier extraction of accumulated organics and less interference from manufacturing contaminants in the triolein are compensating advantages. Both devices are extremely sensitive, being capable of accumulating readily-detectable burdens of PAH from intermittent exposures at the low ng/g (part per trillion) level (Holfelt 1998, Luellen 1999).

We deployed a network of PEMD samplers in Prince William Sound, Alaska, to assess the availability of hydrocarbons remaining within selected beaches from the 1989 *Exxon Valdez* oil spill. These were deployed in winter and again in spring, on the surfaces and buried within the beaches examined. Two of these beaches were located in an area remote from the spill impact area, and were to serve as controls for comparison. An un-reported diesel oil spill, most likely from one or more fishing vessels, occurred during the spring deployment, and was recorded by our samplers. This spill most likely occurred during the height of fishing activity the week before we retrieved our samplers. This allowed us to compare the surface and subsurface impacts of this spill, the results of which we report here.

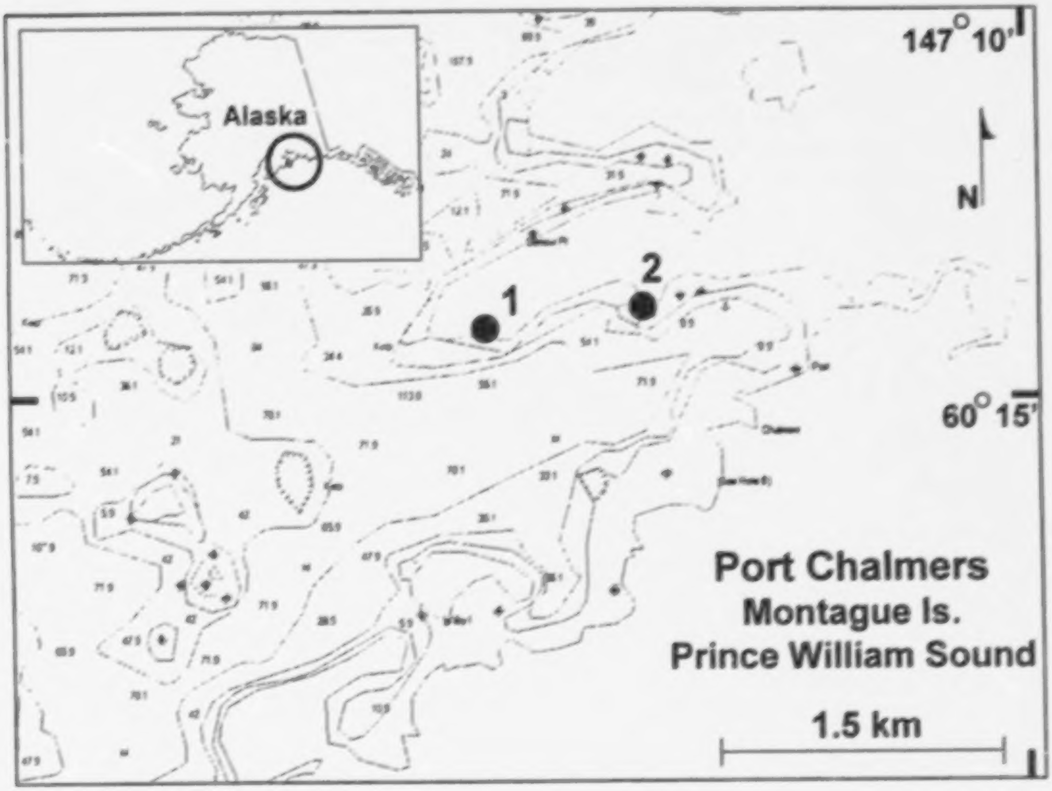

Fig. 1. Map of study area.

Materials and Methods

The study area is located within a small embayment of Port Chalmers, on the northwestern shore of Montague Island in Prince William Sound, Alaska (Fig. 1). This bay is used seasonally as a remote release site for hatchery-reared juvenile chum salmon. Chum salmon (*Oncorhynchus keta*) are reared in marine net-pens during April and May and are then released. Commercial purse-seine boats participate in a hatchery cost-recovery fishery for returning adult chum salmon that opened 31 May – 2 June, and similarly on successive weeks until there were no more fish to recover. The beaches we monitored were about 1 and 2 km from the marine net pen site, on the north shore of the embayment. The beach furthest from the net pens (station 1) consisted of well rounded cobble armor (~0.5 m deep) and sand mixed with cobble underneath (i.e. > 0.5 m depth). Station 2 was at a sheltered rocky site, with bedrock outcroppings interspersed among angular cobble, pebble, granule beaches. Tides are semi-diurnal in PWS, with a maximum vertical range of 6 m. The beach at station 1 is exposed to high-energy waves, while station 2 is more sheltered from the dominant winds and waves from the northeast.

The PEMD samplers consist of low-density polyethylene tubing (98 μm thick × 4.9 cm × 0.5 m; Carls *et al.* 2004) deployed in perforated aluminum containers to protect them from abrasion. These were deployed at station 1 for 28 days beginning 25 February 2002, and at both stations 1 and 2 for 30 days beginning 24 May 2002. At each station, the samplers were placed along two transects (denoted T1 and T2) oriented perpendicular to the shoreline and separated by 3 m, at tidal elevations of 0.5 m, 2.0 m and 3.5 m above tidal datum (mean lower low water; Fig. 2). At each transect and tidal elevation, one sampler was anchored on the surface of the beach. Another was buried at a depth of ~50 cm at station 2, and at a depth of 1 m at station 1 (i.e. 50 cm beneath the interface between the cobble overburden and the cobble/sand layer beneath it). On retrieval, the PEMD containers were disassembled in the field, and the PEMDs were immediately placed into pre-cleaned glass jars and stored at – 20 EC until chemical analysis. Two sediment samples were collected from the 2.0 m tidal elevation of one of the transects at station 2. One of these was from surface sediments and the other from a depth of 50 cm.

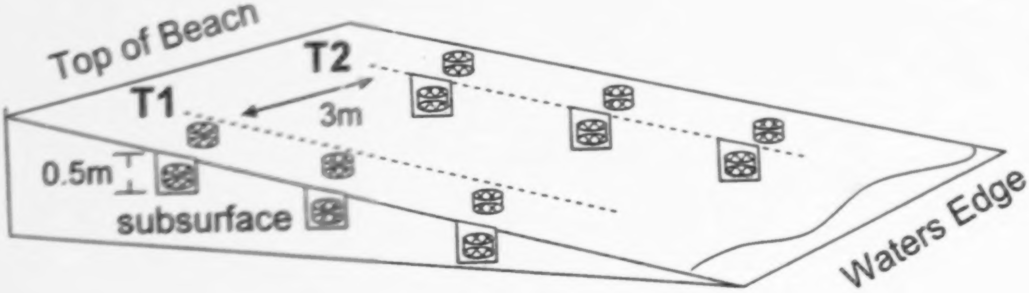

Fig. 2. Schematic of PEMD deployment.

Just prior to analysis, the PEMDs were wiped with cellulose tissue to remove gross surface contamination. The PEMDs containing the accumulated PAH were spiked with a suite of six perdeuterated surrogate PAH standards dissolved in hexane which was allowed to dry, then extracted from the PEMDs with 100 mL of pentane:dichloromethane (4:1 v/v) in a sonic bath for 130 min with a sonic duty cycle of 20 on and 30 min off. Extracts were concentrated to 20 – 30 mL over steam, dried with ~4 g anhydrous sodium sulfate, exchanged into ~2 mL hexane over steam, and passed through a 1.5 g silica gel (5% deactivated) column to remove polar interferences. The PAH in the sediment samples were spiked with the perdeuterated surrogate PAH and then extracted in a Dionex ASE 200 accelerated solvent extractor with dichloromethane at 2000 psi and 100 EC, which with exchanged with hexane over steam, cleaned up with silica gel:alumina column chromatography followed by gel permeation HPLC, and then reconcentrated in hexane. The PAH in the hexane solutions obtained from the PEMDs and the sediment samples were analyzed by gas chromatography/mass spectrometry (Short *et al.* 1996).

The PAH analyzed included both parent homolog and alkyl-substituted naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes (abbreviated as NX, FX, DX, PX and CX, where X = number of alkyl-substituted carbon atoms ranging from 0 to 3 or 4), along with biphenyl (bip), acenaphthene (ace), acenaphthylene (acn), anthracene (ant), fluoranthene (flu), pyrene (pyr), methyl-fluoranthene/pyrene (FP1), benzo-a-anthracene (baa), benzo-b-fluoranthene (bbf), benzo-k-fluoranthene (bkf), benzo-e-pyrene (bep), benzo-a-pyrene (bap), perylene (per), indeno-1,2,3-*c,d*-pyrene (icp), dibenzo-*a,h*-anthracene (dba), and benzo-*g,h,i*-perylene (bgp). Experimentally determined method detection limits, defined as the estimated concentration associated with a 1% probability of type I detection error for these PAH, ranged from 0.006–0.050 :g/PEMD strip, or 0.0006–0.005 :g/g sediment.

Total PAH (TPAH) refers to the sum of the analytes listed in the preceding paragraph. All concentrations are expressed on a dry weight basis.

Results

Winter concentrations of PAH in the PEMDs were very low, with TPAH ranging from 0.012 – 0.112 :g/strip. These concentrations are near the MDLs (method detection limits). These low values were expected, as the site is remote and there was no indication of recent human disturbance in the area.

Spring concentrations of PAH in the PEMDs were much higher ranging up to 722 ug/strip, apparently as a result of a nearby purse seine fishery operation. The PEMD samplers were first retrieved from station 1 at the end of the spring deployment. No visual or olfactory evidence of diesel oil contamination was apparent at this station. However, at station 2, diesel oil sheen and odor were immediately apparent on arrival. At this station, diesel oil sheening and odor was most evident near the mid-tidal elevation of both transects, and extended to the highest tidal elevation of transect T1. Diesel oil was evident in the fine-grained surficial sediments to depths of 2–4 cm in some portions of station 2.

Concentrations of PAH in PEMD strips retrieved from both stations 1 and 2 following the spring deployment confirmed diesel oil contamination, especially at station 2. At station 1, TPAH concentrations ranged from 0.106–1.05 :g/strip for PEMDs deployed on the beach surface, but at two subsurface PEMDs these concentrations were 10–50 :g/strip (Fig. 3a). Concentrations of TPAH ranged from <MDL–0.499 :g/strip at the remaining subsurface PEMD deployments at station 1.

At station 2, TPAH concentrations were greater than station 1, and ranged from 62–722 :g/strip at three of the surface deployments, compared with concentrations ranging from 1.55–2.39 :g/strip elsewhere (Fig. 3b). Concentrations of TPAH were usually lower among the subsurface PEMDs, ranging from 0.098–4.59 :g/strip except at one location where it was 78.4 :g/strip (Fig. 3b).

TPAH concentration of the sediments followed a similar pattern as the PEMDs at the mid-tide elevation at station 2. The TPAH concentration in the surface and subsurface sediments was 0.661 :g/g and 3.39 :g/g, compared with 2.39 :g/strip and 4.59 :g/strip in the respective PEMDs (Fig. 3b).

The distribution of PAH detected in both the PEMDs and in the sediments was clearly consistent with diesel oil (Fig. 4). The alkyl-naphthalenes account for most of the PAH, with smaller contributions from the alkyl-fluorenes, alkyl-phenanthrenes and alkyl-dibenzothiophenes. The absence of PAH with molecular masses greater than the alkyl phenanthrenes was consistent with truncation of the PAH suite characteristic of crude oil by distillation. The preponderance of the naphthalenes, which are among the most volatile of the PAH, was consistent with a recent (~1 week old) diesel oil spill.

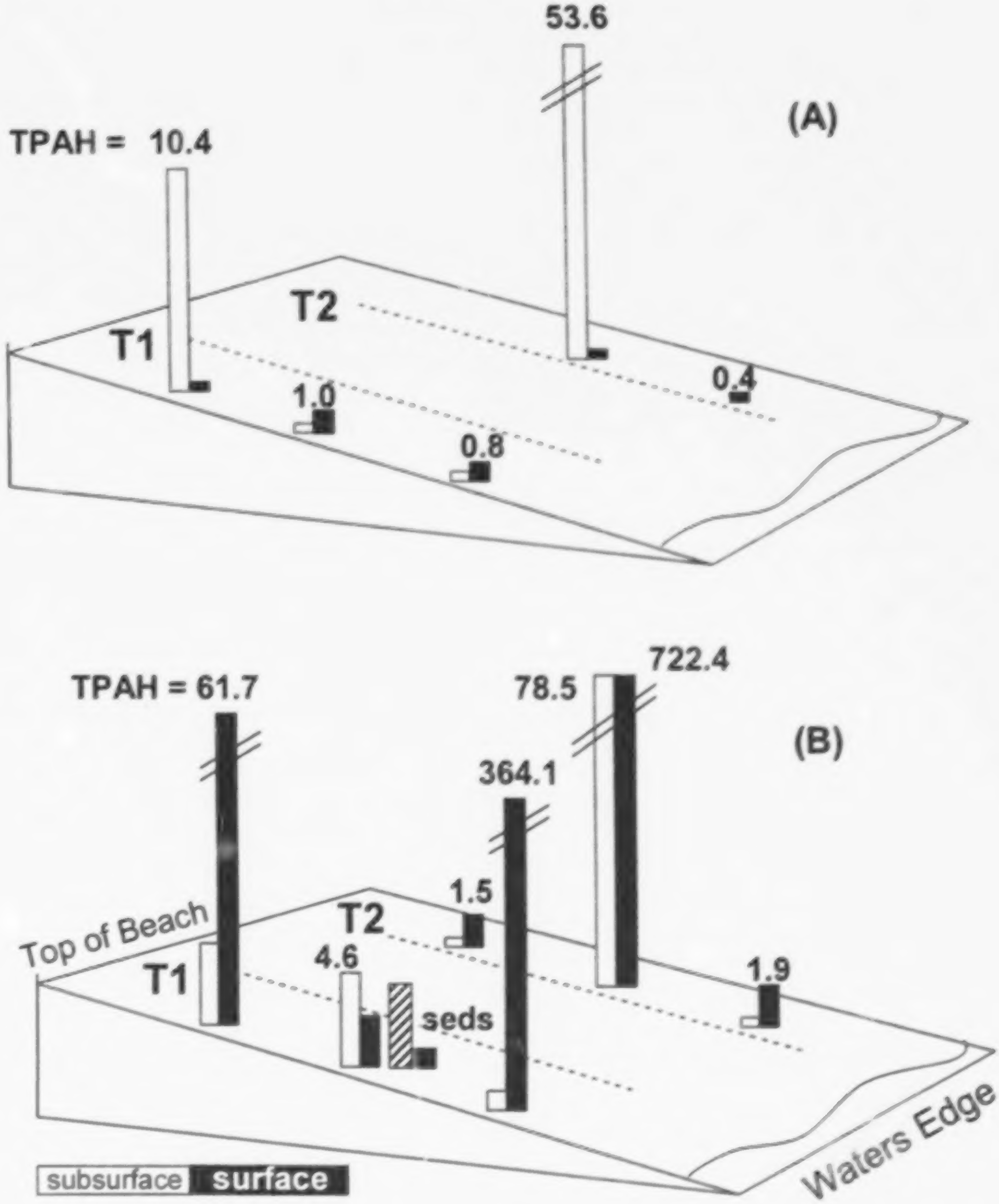

Fig. 3. Total PAH (TPAH = μg/strip) in PEMDs deployed during spring at station 1 (A) and 2 (B).

Discussion

The fact that diesel oil was not apparent to the senses at station 1 attests to the utility of these devices as monitoring tools. Had the extent of this oil spill been based only on evidence available to the senses, the impacted area of beach would have been erroneously concluded to have been much smaller. This suggests that deployment of PEMDs as PAH monitors after an oil spill may help to delineate the magnitude of the impacted area more accurately as well as documenting the persistence over time. Further, absorption into the membranes also indicates their bioavailability into fauna.

A very rough estimate of the amount of diesel oil spilled can be made from the extent of beach contaminated. The presence of diesel at both stations 1 and 2, separated by ~1 km, indicates shoreline contamination spread across at least this distance. If it is assumed that the 1 km shoreline were contaminated throughout the intertidal (about 20 m from the extreme of upper to lower intertidal) with a 1 mm thick layer of diesel oil, an oil volume of 20 m^3 results. This suggests that the amount of diesel oil spilled is probably at least on the order of cubic meters. This is consistent with the size of a fuel tank on one of the seine vessels involved with the fishery, suggesting a ruptured tank as a possible source.

Accumulation of a definite PAH suite characteristic of diesel oil in the subsurface PEMDs at station 1 confirms the ability of stranded diesel oil to permeate porous beaches to substantial depths. Note that accumulation of these PAH was not induced by disturbance of the beach sediment during initial deployment, because the spill occurred afterward. The absence of correspondingly high PAH levels in the PEMDs on the surface at station 1 is because of the large particle-size sediments on this more exposed beach. Diesel oil initially stranded on cobbles would readily percolate to the underlying finer-grained sediments on an outgoing tide, becoming trapped there by capillary forces. Once trapped, subsequent migration of the oil, or of PAH dissolved from the oil, would either follow interstitial groundwater flow, or else become rapidly diluted by seawater once re-emerging at the surface.

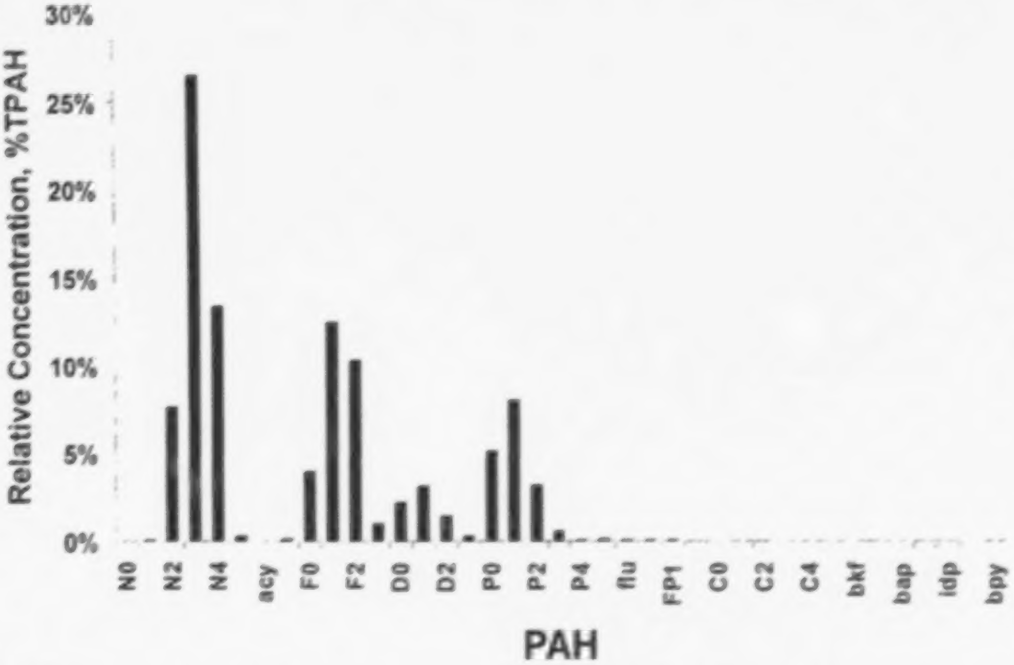

Fig. 4. Distribution of PAH in PEMDs deployed at station 1 and 2 during spring (see methods for PAH abbreviations).

The higher PAH levels of the PEMDs located at station 2 reflect the greater shoreline oiling there. The finer grained surficial sediments at station 2 compared with station 1 were more effective at retaining diesel oil that had percolated into them. The high concentrations of diesel PAH in subsurface-, and especially the surface-deployed PEMDs at station 2 are corroborated by the analyses of sediments, as well as of interstitial water collected at this site, where TPAH concentrations were 1.00 :g/L and 6.77 :g/L at the mid-tide elevations (Payne *et al.* in review). As at station 1, the elevated PAH in the subsurface PEMDs at station 2 confirm the penetration of PAH derived from the diesel oil at least to 0.5 m below the surface. Contamination of the beach sediments to this depth suggests that the diesel oil may persist for longer than a few days or weeks, because flushing of subsurface sediments is a much less effective process for removing PAH compared with surficial sediments in this region. Oil residues from the 1989 *Exxon Valdez* oil spill have persisted in subsurface sediments for over a decade (Short *et a*l. 2004).

Comparison of results for the PEMDs, sediments, and interstitial water samples from the mid-tide elevations at station 2 illustrates the great sensitivity of the PEMDs as environmental hydrocarbon monitors. The TPAH burden of the subsurface PEMDs exceeded MDLs by factors of more than 250, whereas the sediments and interstitial water samples were only 40–60 times greater. Their high sensitivity and relatively low cost make PEMDs very attractive alternatives for monitoring oil pollution persistence and delineating geographical magnitude on porous beaches.

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Development of water quality objectives and management systems for the lower Athabasca River in the oil sands area. L. Noton[1] and P. McEachern[2]. [1]Alberta Environment, Water Quality, Edmonton, AB; and [2]Alberta Environment, Regional Environmental Management, Edmonton, AB.

The oil sands industry is expanding at a high rate along the lower Athabasca River in northeastern Alberta. Environmental concerns include potential effects on water quality of the river. Although wastewaters reaching the Athabasca River are presently not having significant effects, wastewater releases may increase markedly as the industry expands. The Cumulative Environmental Management Association (CEMA) is a multi-stakeholder group addressing environmental management in the Athabasca oil sands area. CEMA established a water quality task group to look into the issue of water quality protection and management, in view of the predicted increase in wastewater releases. The task group has defined its purpose as 'to develop and recommend water quality objectives and management options on the lower Athabasca River'. The group is following a four-part process for this: (1) Define the problem, (2) Set goals, (3) Measure performance, and (4) Manage and adapt. Under (1), approximately 35 water quality variables of potential concern have been conservatively identified in the oil sands area. Under (2), the group has identified the uses of water it wishes to protect, has stated its intent to prevent degradation of water quality wherever possible, has reviewed available water quality guidelines, and has drafted a plan for developing site specific water quality objectives. Under part (3), performance will be estimated using water quality models to simulate full-development scenarios. River mixing studies and model upgrading have been done to prepare for this. Management (part (4) will be designed for potential impacts forecast by the modelling work.

Ecotoxicological assessment of using oil sands coke in aquatic reclamation strategies. A.J. Squires and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Two companies currently mining the Athabasca oil sand deposits in northern Alberta are Syncrude Canada Ltd. and Suncor Energy Inc. As part of the oil upgrading process, each company produces over 2,000 tons of coke waste product each day. Coke has been proposed for use as an amendment in wetland reclamation strategies due to its high organic carbon content. However, coke contains various metals and organic compounds, which may have the potential to leach out after being submersed in water. A series of leaching/weathering experiments were conducted on coke obtained from both mining companies to determine their potential to leach out contaminants while exposed to various environmentally relevant overlying water conditions. These included high (7.9 mg/L) and low (0.9 mg/L) dissolved oxygen content, pH levels of 5, 7.5 and 10, and several (5) freeze-thaw cycles. During each experiment, samples of the overlying and coke interstitial waters were taken for chemical analysis. Afterward, 10-day old larvae of the macroinvertebrate, *Chironomus tentans*, were exposed to both coke types along with their overlying leachates, and to the leachates separately, to differentiate between effects from the solid coke and the leachate. None of the leachates showed a significant impact on survival or growth (measured as dry weight) of *C. tentans*, but some effects were observed following exposure to the "weathered" coke. The results from these experiments will help determine the potential of coke to adversely affect aquatic organisms if used as an uncovered capping option during an aquatic reclamation program at the Athabasca oil sands.

Using stable carbon isotopes to monitor bacterial degradation of naphthenic acids and polycyclic aromatic hydrocarbons. P.P. Videla, A.J. Farwell, B.J. Butler, V. Nero and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The extraction of bitumen from the oil sands in northern Alberta produces large amounts of process-affected water. The tailings are placed into settling ponds prior to reclamation and consist of sand, clay, unrecovered bitumen, naphthenic acids (NAs), and polycyclic aromatic hydrocarbons (PAHs). NAs are a complex mixture of carboxylic acids that have been analytically characterized by C numbers (C5-C33) and Z families (Z=0, linear; Z=-12, 6 rings). The NAs are found in large quantities in reclaimed aquatic environments and to some extent are biodegradable, which could reduce their toxicity. Field studies suggest that stable carbon isotopes may be used to better understand carbon flow from oil sands organic compounds to aquatic organisms. Isotope analyses may provide a tool to define exposure to oil sands constituents as previous work has shown the ^{13}C depletion of benthic invertebrates *process-affected material containing NAs and PAHs. To understand* the cycling of oil sands carbon sources through aquatic foodwebs in terms of C isotopes, we need to examine the isotope fractionation associated with degradation of complex mixtures of NAs and PAHs. Laboratory biodegradation studies were conducted using oil sands extracted PAHs and NAs and commercial NAs that differ in C number and Z family distributions. Concentrations of NAs or PAHs in modified Bushnell-Haas medium were inoculated with a NA-degrading enrichment culture, derived from NA-contaminated subsoil. The concentration and $\delta^{13}C$ values of DIC, DOC and POC were measured at various intervals during the course of the experiments.

Microbial carbon sources in boreal wetlands. C.A. Daly and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

My research examines the role of heterotrophic microbial producers as decomposers of organic matter, the carbon source supporting them, and their role at the base of the aquatic food web in boreal wetlands. Stable isotope analysis will assess the relative importance of diverse sources of carbon (wetland detritus, phytoplankton, and anthropogenic sources of organic matter) to microbial production. Constructed wetlands are a remediation strategy following oil sands extraction in the Athabasca oil sands region of north-eastern Alberta. I will resolve the extent to which residual bitumen in young oil sands-affected wetlands serves as a surrogate for allochthonous carbon sources that form the base of the food web in naturally formed wetlands. As part of a collaborative effort to understand energy flow in natural and constructed wetlands, I will: (1) estimate the contributions of different carbon sources to microbial biomass using stable isotope analysis, (2) estimate planktonic and benthic microbial biomass (epifluorescence direct count microscopy) and production (leucine incorporation into microbial protein), and (3) respiration losses (CO2 elution) in wetlands of contrasting ages and histories. Measures of microbial biomass and production rate indicate how much carbon is potentially available to higher trophic levels. However, it is not clear how microbial production becomes allocated as detrital material, as food source for zooplankton and zoobenthic communities, and as carbon respiration loss to the atmosphere. All three compartments have significant implications for boreal wetland reclamation strategies in disturbed areas.

The role of stress in ecosytem properties: the benthic invertebrate community of the Alberta oil-sands wetlands. C.M. Wytrykush and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

Environmental pressures cause stress, which is a measurable change of an organism's steady state. Stress from natural or anthropogenic environmental pressures can affect individuals, populations and communities. Stress can affect ecosystem processes and this can be evaluated by examining changes in diversity. Stress can also affect ecosystem functions such as primary productivity. The purpose of my research is to examine environmental stress response of the benthic macroinvertebrate community (primarily Diptera) in natural and constructed wetlands in the oil-sands region of northeastern Alberta. These wetlands contrast in condition (oil-sands process affected vs. natural), level of sediment organic content (low vs. high) and age (young vs. mature). Oil-sands process materials are enriched with several types of compounds including sulphate ions, ammonia, polycyclic aromatic hydrocarbons(PAHs), and naphthenic acids. In high concentrations, these compounds are potentially toxic to aquatic invertebrates, and are considered here as environmental stress. I am determining primary and secondary production in stressed and unstressed wetlands, and calculating benthic macroinvertebrate diversity to determine relationships between productivity and diversity. I will also determine food chain length by measuring maximum trophic position, and ascertain whether food chain length is influenced by energetic constraints, ecosystem size and ecosystem stressors I have tested whether benthic invertebrate taxon richness is controlled by primary productivity. Preliminary results indicate that there is a significant positive relationship between taxon richness and primary productivity. Analysis is ongoing to investigate this relationship in more detail and to determine the relationships between primary productivity, secondary productivity and invertebrate diversity and how these are influenced by stress.

Histopathological effects of naphthenic acids and salinity to yellow perch, *Perca flavescens*. V. Nero[1], A.P. Farwell[1], L.E.J. Lee[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Previous quantitative histopathological work has shown that experimental aquatic reclamation sites constructed from materials created during oil sands mining in northeastern Alberta, Canada induced greater gill (cell proliferation) and liver (degenerative) tissue alterations in a native fish species, yellow perch (*Perca flavescens*). As these reclamation sites contain a complex mixture of elevated levels of naphthenic acids (NAs) and salinity, and also low levels of polycyclic aromatic hydrocarbons, the objective of this study was to examine the independent and interactive effects of NAs (extracted from process-affected water) and salinity (sodium sulphate) to yellow perch to determine if these agents were responsible for the tissue alterations observed in fish exposed to whole process-affected water. Following three-week laboratory exposures, the gills of yellow perch exhibited high levels of cellular proliferations while the examination of liver tissue showed very little change at the level of NAs (1.7 mg/l) tested in this study. Furthermore, exposures containing mixtures of NAs and low levels of salinity (1 g/l) significantly yellow perch gills. This study has shown that the histopathological alterations of the gills observed in yellow perch residing in aquatic reclamation sites can be directly attributed to the combination of NAs and low levels of salinity. The minimal effects of NAs and salinity to yellow perch liver in the laboratory exposures suggests a greater sensitivity of fish gills to NAs or a possible reduction in the exposure of NAs to the liver resulting from the gill alterations observed in exposed fish.

The use of stable carbon isotopes to examine the degradation of naphthenic acids.
A.P. Farwell, B.J. Butler, V. Nero, P.P. Videla and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The extraction of bitumen from the Athabasca oil sands deposit in northern Alberta, Canada generates large volumes of process-affected water containing elevated levels of naphthenic acids (NAs), polycyclic aromatic hydrocarbons and residual bitumen. Naphthenic acids are acutely toxic constituents that are biodegraded and become less toxic in reclaimed aquatic systems. This study uses stable isotopes to characterize the degradation of oil sands constituents to improve the understanding of the cycling of carbon through aquatic foodwebs and to develop carbon isotope analyses as a tool to define exposure of aquatic organisms to oil sands constituents influenced by natural or anthropogenic sources. NAs represent a complex mixture of chemicals characterized by C numbers ranging from C5-C33 and Z families, descriptive of ring structures, ranging from 0 (linear) to -12 (6 ring). To better understand the isotope fractionation associated with the biodegradation of NAs in the field, different NA mixtures were compared in terms of their NA profile and C isotope values. As NAs are degraded, the proportion of NAs within classes of C numbers (C5-C13, C14-C21, C22-C33) and Z families (0 to -4, -6 to -12) changes. NA mixtures ranged in $\delta^{13}C$ values from -27 to -8‰.

Differences in the $\delta^{13}C$ values were related to changes in the proportion of C5-C13 and C14-C21 classes such that high C5-C13:low C14-C21 mixtures were the most ^{13}C depleted. Differences in the $\delta^{13}C$ values of the various NA mixtures suggests that stable isotopes may be useful to examine the degradation of NAs.

Rapid toxicological evaluation of oil-sands' process-affected water using fish cell lines. V.R. Dayeh[1], V. Nero[1], A.P. Farwell[1], D.G. Dixon[1], N.C. Bols[1] and L.E.J. Lee[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Large amounts of tailings and process-affected waters are associated with the extraction of petrochemicals from the oil sands of Athabasca, Alberta. Various mixtures of oil-sand's process-affected waters (OSPAW) are being evaluated in reclamation ponds for its eventual release to the environment. Acute and chronic toxicity tests have been developed with various model organisms to evaluate the safety of these reclamation ponds. A rapid fluorometric assay using fish cell lines was developed to assess the acute toxicity of OSPAW. Impairment in several cellular functions including cell membrane integrity, mitochondrial and cytoplasmic activity, and lysosomal function were evaluated using a fluorescence microplate reader. Increasing concentrations of OSPAW from 15-100% from several experimental reclamation ponds were directly evaluated on cells in culture without prior extraction procedures. This evaluation was done in blind by the experimenter and a direct correlation to naphthenic acid (NA) content and toxicity was observed. NAs are complex mixtures of saturated carboxylic acids found at high levels in OSPAW. This approach could provide a reference base for rapid assessment of NA toxicity as well as from its fractions and mixtures to biomonitor oil constituents and contribute to environmental risk assessment.

Chronic toxicity study on snowcrab exposed to drilling fluid being used on the Grand Banks. C.D. Andrews[1], B. French[2], L. Fancy[1], J. Guiney[1] and J.F. Payne[1]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NL; and [2]Oceans Ltd., St. John's, NL.

From a socioeconomic standpoint, the major concern about oil development in the offshore is the potential for impacts on fisheries. The crab fishery is presently the most important fishery in the NF region. It is also one of the major fisheries in the Gulf of St. Lawrence, another promising area for oil development. An aliphatic hydrocarbon based drilling fluid is being used on the Grand Banks. Oils enriched in aliphatics are generally considered to have a very low toxicity potential and this has been supported by a number of acute toxicity studies carried out on different species in our laboratory over the past few years. It is further noted that aliphatic hydrocarbons are commonly used in medicinal and cosmetic formulations. A chronic toxicity study of a pilot nature has now been carried out on snowcrab. Chronic toxicity studies are needed for assurance or to possibly uncover unrealized toxicity potential. Crabs were exposed per os to drilling fluids resulting in rather large concentrations of aliphatic hydrocarbons in hepatopancreatic tissues, in the 1000–2000 mg/kg range. Animals were sacrificed after a month and the indices examined included selected hepatopancreatic enzymes, hematology, and detailed tissue histopathology. Most indices remained unaffected with no evidence for histopathological change. Given the very high hydrocarbon concentrations to which the animals were exposed, results support the hypothesis that the aliphatic hydrocarbon based drilling fluid being used on the Grand Banks should pose little or no risk to snowcrab. (The study was supported in part by PERD).

Pilot study on histopathological abnormalities in winter flounder in association with Sydney tar ponds. A. Mathieu, R. Soper and B. French. Oceans Ltd., St. John's, NL.

Polycyclic aromatic hydrocarbons, metals and other compounds were released over a 90-year period or so from coke ovens into the Muggah Creek area of Sydney Harbour. The area is presently recognized as one of the most toxic sites in Canada. Early warning indicators of effects on fish health have the potential to act as sensitive tools for assessing complex pollution conditions and overall environmental quality. The health of fish or other wildlife in a contaminated area also has potential to provide linkages to questions concerning human health. Earlier studies by the Department of Fisheries and Oceans (DFO) indicated that winter flounder in the area had elevated levels of liver enzymes. Histopathology can reflect the integrated effects of contaminants over time and is increasingly being used in national and regional monitoring programs. We have now carried out a preliminary study for histopathological abnormalities in liver tissues of 50 winter flounder from the mouth of South Arm of Sydney Harbour. A number of pathologies, including tumorous type lesions, were found in flounder from the area. The most common lesion observed was hydropicvacuolation which has also been reported to be the most common lesion in urban and industrialized areas near Boston and New York. Overall, this preliminary study suggests that waters in the area are degraded to a sufficient degree to affect the health of flounder. A more comprehensive study including analyses of larger numbers of fish and sampling closer to Muggah Creek would provide a better ecotoxicological picture of the area. Such an ecotoxicological baseline could also be valuable for assessing "before" and "after" conditions for any remediation program. (The study was supported in part by TSRI funds from Drs. Ken Lee and Jerry Payne, Department of Fisheries and Oceans).

The environmental impact factor; a risk assessment tool for the offshore oil and gas industry validated and calibrated with effect monitoring data. M. Smit, C. Karman and R. Jak. Netherlands Organization for Applied Scientific Research; Environment, Energy and Process Innovation; Den Helder, The Netherlands.

For the assessment of impacts (EIA) on the marine environment related to offshore activities of the oil and gas industry, Environmental Risk Assessment (ERA) and Environmental Effect Monitoring (EEM) are commonly applied as two independent tools. In Norway a development has started to integrate ERA and EEM for the environmental management of offshore releases. One of the recently developed ERA tools for offshore operations is the Environmental Impact Factor (EIF). The concept is based on a 3D-dispersion model for produced water combined with a straightforward PEC-PNEC approach. The EIF concept is implemented in a risk based management tool for environmental care related to produced water releases and is acknowledged by the Norwegian authorities to be used for defining platform specific mitigating measures. Recently a project was raised to develop an EIF for drilling discharges as well. It is the objective that this EIF will be used for managing environmental risks in the water column and sediment related to the drilling process, including toxic and non-toxic disturbances. To reduce the uncertainty both EIF models will be validated (and partly calibrated) with data from EEM. This facilitates the comparison of ERA and EEM and improves the EIA. In this paper the basic concepts for the EIF for produced water and drilling discharges will be presented in relation to a general framework for EIA. Emphasis will be put on the links between EEM and these ERA models. When fully developed, the EIF approaches will totally integrate EEM studies and form an integrated framework for EIA.

Polycyclic aromatic hydrocarbons in Northwest Atlantic finfish: available and needed knowledge for monitoring. J. Hellou[1], J. Leonard[1], T.K. Collier[2], and F. Ariese[3]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [2]Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA; and [3]Department of Analytical Chemistry and Applied Spectroscopy, Free University of Amsterdam, Amsterdam, The Netherlands.

There is a widespread interest in assessing and monitoring exposure to polycyclic aromatic hydrocarbons (PAH) in humans, other vertebrates and invertebrates to prevent associated potential toxic risk that can cover a range of effects. Although these chemicals degrade over time, depending on their source and structure, they can persist long enough and be in a high enough concentration to have a potential toxic risk associated with exposure (van der Oost *et al.* 2003). More studies involving invertebrates have examined bioaccumulation than biotransformation, although metabolism has been shown to take place in a few species (Stromberg *et al* 1999). Vertebrates have active mixed function oxygenase enzymes that allow biotransformation to take place more readily than in bivalves. The fate of the oxidation products is of interest because they are linked to the formation of DNA-adducts associated with carcinogenic effects (Akcha 1999, Aas *et al.* 2000, Johnson *et al.* 2002).

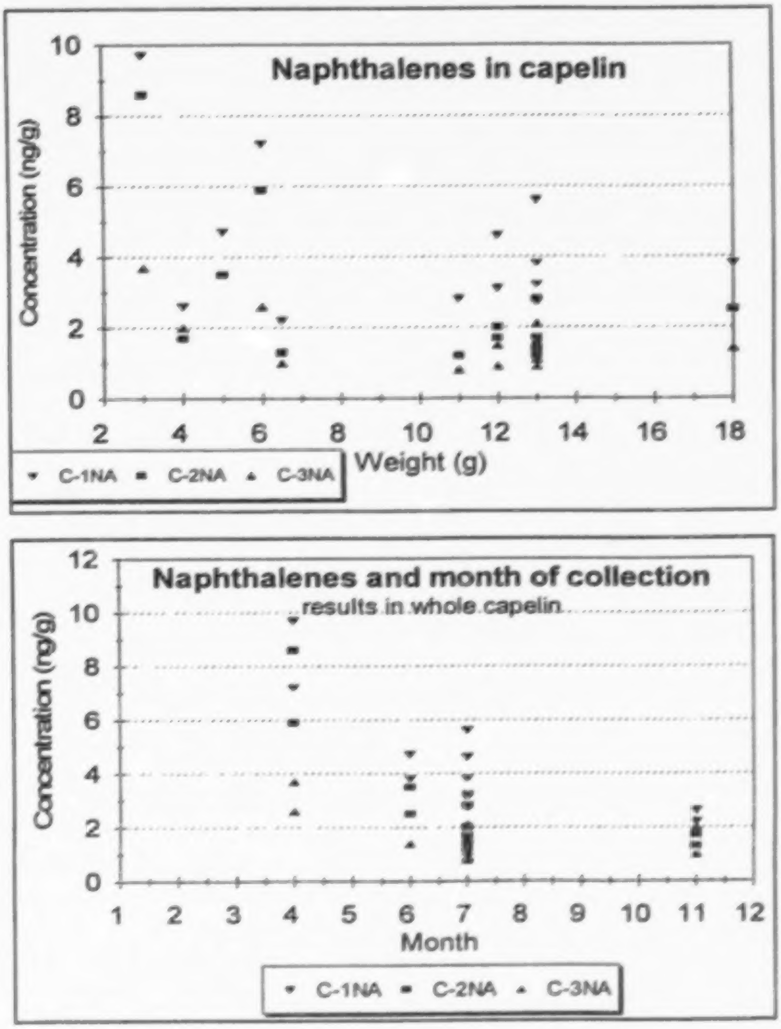

Fig. 1. Concentrations of C-1, C-2 and C-3 naphthalenes (NA) determined in whole fish and compared to the weight of fish and month of collection.

Monitoring can involve analysing the exposure media or the exposed organisms for various chemical, biochemical or biological endpoints. Depending on the animals studied, an organism, a tissue or pools of either can be targeted. Variability in uptake and examined endpoints will be higher in short-term compared to long-term experiments, while it is also expected to be lower if organism related variables are restricted, such as age or size (Bignert *et al.* 1993).

Our study examined PAH concentrations in small finfish (<30 cm) represented by capelin, sand lance, American plaice, yellowtail flounder and herring collected opportunistically in various Northwest Atlantic Fisheries Organisation (NAFO) divisions. Variables covered in the offshore sampling were pool size, size differences within and between species, lipid content and location. Results were obtained on whole finfish, with a smaller number of samples comparing concentrations in internal organs to the rest of the carcass. Bioaccumulation trends pointed to two exposure routes, respiration and feeding, and two sources, combustion and fossil fuel. An example of differences observed in capelin according to collection time and size of fish is illustrated in Fig. 1. Alkylated naphthalenes present in all samples would have been taken up by respiration and are derived most likely from underwater petroleum seeps. Higher concentrations of three alkylated naphthalenes were present in smaller than larger fish. The relative concentration of these three PAH was C-1NA > C-2NA > C-3NA correlating with exposure to fresh oil, rather than weathered. Internal organs displayed similar levels of PAH to those in muscle tissue (rest of carcass), and interpreted as exposure having a dietary contribution. This is a logical conclusion since if fish are exposed through respiration, then their prey are also exposed, hence there are PAH circulating in the food chain. Since PAH are biotransformed by fish, following up on exposure is more challenging than it is with a number of invertebrates. The dietary proportion of exposure increases with the molecular size of a PAH, since these are more hydrophobic than smaller PAH.

Parental PAH including phenanthrene, fluoranthene, pyrene and chrysene were more abundant in sand lance and can not be associated with atmospheric transport, but rather with burrowing/feeding on weathered oil or combustion material. Measurements were carried out before the development of the Hibernia oil fields and the observed tissue concentrations represent baseline levels for future comparison. Biotransformation products were not measured, but are needed to better assess future exposure and effects, especially with long term exposure to produced water, where PAH could potentially be associated with toxicity (Stoemgren *et al.* 1995).

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The toxicity of photomodified polycyclic aromatic hydrocarbons and dibenzothiophene congeners to Japanese medaka embryos. A.P. Farwell, P.S. Bal, M. Croft and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Alkylated polycyclic aromatic hydrocarbons (alkyl-PAHs) are naturally occurring compounds found in process-affected water following the extraction of bitumen mined from the Athabasca oil sands in northern Alberta, Canada. Previous studies have shown that the base-neutral hydrocarbon fraction of process-affected water (rich in alkyl-PAHs) significantly increased the frequency and severity of blue sac disease (BSD) symptoms, while reducing larval fork length at hatch in Japanese medaka (*Oryzias latipes*) 18 day early-life stage bioassays. The C2 - substituted dibenzothiophenes (DBTs) were found to be the most common hydrocarbons of the parent and methylated US EPA PAH priority pollutants (16) measured in the extract. The oil sands PAH extract, dibenzothiophene and dimethylated dibenzothiophene were exposed to simulated solar radiation (SSR) to determine the effects of UV exposure on the toxicity of a complex PAH extract and individual constituents known to be elevated in oil sands material. The Japanese medaka 18 day early-life stage bioassay protocol was modified from previous studies in order to accommodate testing for the changes in toxicity due to photomodification and photosensitization. The oil sands PAH extract, exposed to simulated solar radiation (SSR) for 1 and 4 days, showed decreased toxicity to Japanese medaka embryos relative to the non-photomodified extract. Changes in toxicity associated with the photomodification and photosensitization of the PAH extract and dibenzothiophene congeners will be discussed in terms of the implications of PAH-UV exposure in reclaimed and natural aquatic environments in the region of the Athabasca oil sands deposit.

Effects of petroleum coke on development of invertebrate and macrophyte communities in constructed wetlands. L.F. Baker[1], J.J.H. Ciborowski[1] and M.D. MacKinnon[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; and [2]Syncrude Canada Ltd., Edmonton, AB.

Syncrude Canada Ltd. of Fort McMurray, Alberta currently produces approximately 2 million tonnes of coke per year as a by product of oil sands mining. One storage proposal is to use the stockpiled coke to stabilize clay-dominated mine tailings in constructed wetlands currently being studied as an option for landscape reclamation. However, there is limited knowledge of the toxicity of coke and its associated leachates (trace metals and polycyclic aromatic hydrocarbons) to aquatic biota. We studied *in situ* effects of petroleum coke on the invertebrate and macrophyte communities of constructed wetlands, and whether or not adding a surface layer of peat would affect community establishment. Treatments consisted of 40 cm diameter x 10 cm thick layers of coke, sand, or natural sediment, with or without a 2 cm thick topping of peat spread on the treatment substrate. Treatments were placed in 3 constructed wetlands in August 2002, June 2003, and August 2003. All test patches were collectively sampled in August 2003. Plant cover(%) on each patch was visually estimated. Zoobenthos were sampled from 325 cm2 colonization tiles that were in place for 8 days. Preliminary results indicate that after 1 year invertebrate and macrophyte communities are not negatively affected by the *in situ* treatments of coke, and peat has no ameliorating effect.

Pharmaceuticals / Pharmaceutiques

Session co-chairs: K.A. Kidd and B.K. Burnison

Partitioning and biodegradation: determining the fate of phenolic estrogenic compounds in marine sediments. B. Robinson[1], M. Langille[2], J. Hellou[3]. [1]Department of Oceanography, Dalhousie University, Halifax, NS; [2]Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

In recent years, advancements in analytical technology have led to the detection of trace concentrations (ng/L) of chemicals that had previously been undetected in aquatic environments. These chemicals, known as endocrine disrupting compounds (EDCs), are capable of interfering with the natural functioning of the endocrine system. A specific group of EDCs known as estrogenic compounds are capable of mimicking the natural endogenous hormone estradiol, and in turn causing specific changes in the sexual development of many aquatic organisms (Arcand-Hoy *et al.* 1998). A variety of compounds have been identified as potential estrogenic compounds, including natural estrogen hormones, and many anthropogenic compounds such as pharmaceuticals and industrial chemicals.

An important aspect of research in the field of estrogenic compounds is to examine their behaviour and fate once they are released in the environment through sewage effluents. To date, the majority of research on this topic has occurred in freshwater environments (Lai et al. 2000, Holthaus *et al.* 2002, Jürgens *et al.* 2002), while research in the marine environment is limited (Ying and Kookana 2003). The goal of this study is to examine the behaviour and fate of several estrogenic compounds in the marine environment. To accomplish this goal, an analytical method has been developed to detect several phenolic estrogenic compounds in marine samples. This method was applied to samples collected from Halifax Harbour. These samples were also used to conduct experiments to in the laboratory to examine the sorption and biodegradation of these compounds in marine seawater and sediment.

Three phenolic estrogenic compounds were selected as the target compounds for study. The steroid 17â-estradiol is a natural hormone that is excreted mainly by females and is found in both treated and untreated sewage effluents. Ethynylestradiol is a synthetic estrogen and is the active ingredient in oral contraceptives. Bisphenol-A is a synthetic industrial compound used in the manufacture of various plastic products.

Halifax Harbour, like many other marine inlets, has been used for the disposal of human and industrial wastes for over 200 years. There are currently 44 sewer outfalls and 10 fluvial drainage systems that release over 180,000 cubic meters of raw untreated sewage per day (Buckley *et al.* 1995), as well as input from two STPs. Due to this large volume of sewage discharge, Halifax Harbour represents an ideal location to study estrogenic compounds in the marine environment. Locations for the sampling of water and sediment from the Harbour were selected based on their proximity to known sewage outlets.

The analytical method for the detection of the target compounds in seawater was based on previously established methods for freshwater analysis. Seawater samples (1-5 L) were collected, acidified and filtered to remove particulate matter. The target estrogenic compounds were then extracted from the water and concentrated using solid phase extraction. These extracts were fractioned to help remove background interference before the target compounds were identified and quantified using either high performance liquid chromatography (HPLC) with fluorescence detection or gas chromatography/mass spectrometry (GC-MS).

The sorption and biodegradation of the target compounds in different marine sediments were studied using a series of laboratory experiments. Mixtures of seawater and sediment from different locations in Halifax Harbour were spiked with the target compounds. Samples were taken from this mixture at predetermined time intervals over a period of two weeks to determine the concentrations in the water and sediment phases. The effect of different sediment microbial communities on the biodegradation rates of the compounds was examined by comparing sewage contaminated samples collected from Halifax Harbour to a clean reference site in the Bay of Fundy.

Preliminary HPLC results from the analysis of seawater samples collected from two locations near sewage outlets in Halifax Harbour indicate that Bisphenol-A and estradiol were present at concentrations ranging from 20-100 ng/L. These concentrations are higher than expected for estradiol based on previous studies in freshwater environments impacted by sewage pollution, where concentrations ranged in the 0-10 ng/L range (Snyder *et al.* 1999, Ternes *et al.* 1999). Future analysis of water samples will be conducted using GC-MS and LC-MS for more advanced identification and lower detection limits for quantification.

The sorption and biodegradation experiments were conducted using seawater and sediment collected during the summer of 2004 from two sites in Halifax Harbour, as well as a reference site. The properties of the water and sediment collected from these sites is summarized in Table 1. When compared to the reference site, the sediment samples collected from Halifax Harbour were high in organic carbon content, and the water samples also had high total and fecal coliform counts which indicates sewage contamination.

	Sediment Properties		Seawater Properties	
	T.C. (%)	**O.C. (%)**	**Total Coliforms**	**Fecal Coliforms**
Halifax Harbour Site A	5.53	5.19	92,000 per 100ml	24,000 per 100ml
Halifax Harbour Site B	7.17	6.76	1.6 million per 100ml	540,000 per 100ml
Reference Site	0.88	0.69	N/A	N/A

Table 1: Properties of the sediment (% Total Carbon, % Organic Carbon) and seawater (Total and Fecal Coliforms) used in the sorption and biodegradation experiments

The preliminary results for the sorption and biodegradation of estradiol in the seawater/sediment mixtures are shown in Fig. 1 for all three sites. The aqueous concentration of estradiol decreased significantly in the first 48 h of the experiment, and by the end of two weeks estradiol was not detected in the aqueous phase (Fig. 1a). The sediment concentrations of estradiol were highest at the beginning of the experiment, and after two weeks all the estradiol had been degraded (Fig. 1b). The higher concentrations of estradiol in the Halifax Harbour sediments indicates that sorption onto these organic rich sediments plays an important role in removing these compounds from the aqueous phase (Lai *et al.* 2000). A strong correlation exists between estradiol sorption and organic carbon content of sediments (Fig. 2). The complete biodegradation of estradiol in the water/sediment mixture from all three sites observed after two weeks is similar to the biodegradation rates reported in previous studies (Jürgens *et al.* 2002, Ying and Kookana 2003). This indicates that micro-organisms present at the reference site, and in the Halifax Harbour samples, are capable of biodegrading estradiol.

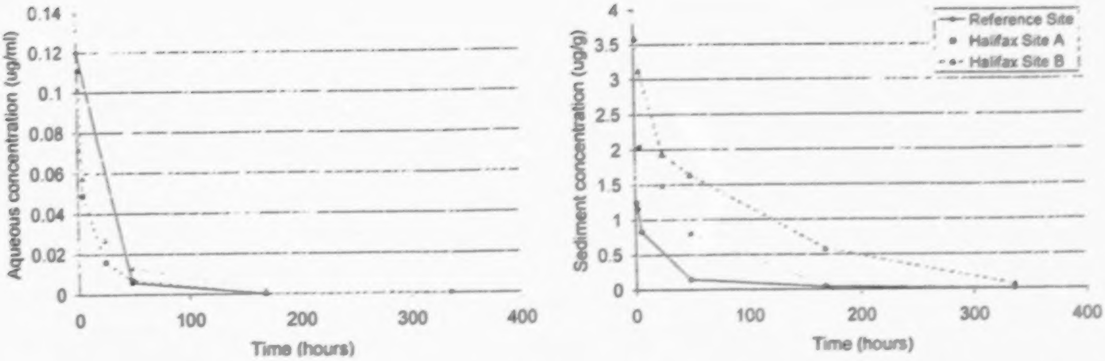

Fig. 1. The change in concentration of estradiol in the (a) aqueous phase and (b) sediment phase during the sorption/biodegradation experiments. The aqueous phase concentration of estradiol decreased significantly in the first 48 h of the experiment, and by the end of two weeks estradiol was no longer detected in the samples from all three sites. The concentration of estradiol in the sediment was initially higher in the samples from Halifax Harbour because of their higher organic carbon content compared to the reference site.

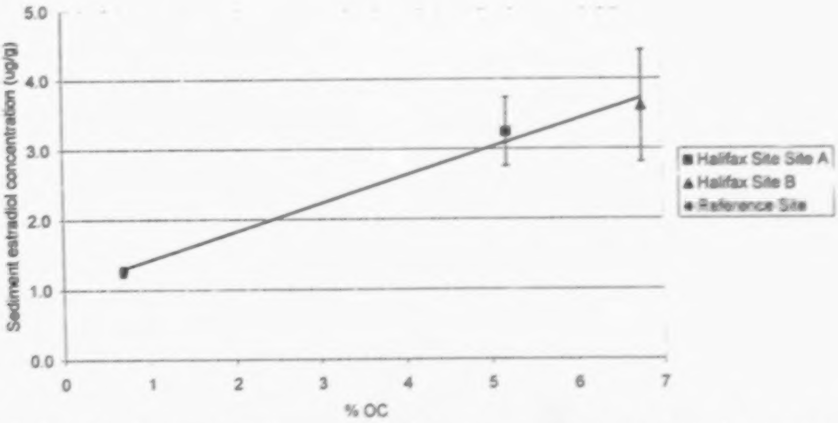

Fig. 2. The relationship between sediment estradiol concentration and organic carbon content of the sediment from the three sampling sites. Significantly higher sorption was observed onto the organic rich sediments of Halifax Harbour compared to the reference site.

Similar trends were observed for the biodegradation and sorption of bisphenol-A and ethynylestradiol (Fig. 3). It can be seen that these synthetic compounds were completely biodegraded after two weeks in both the water and sediment phases. However, these compounds were biodegraded more slowly than estradiol in the aqueous phase, and they were both found in higher concentrations in the sediment. These trends of slower biodegradation and higher sorption for these two synthetic compounds have been reported by previous studies in freshwater environments (Lai *et al.* 2000).

Future research will conduct further sorption/biodegradation experiments using seawater and sediment collected from other locations in Halifax Harbour where the seawater and sediment properties are different compared to the locations previously studied. Experiments will also be conducted using autoclaved samples to study the sorption process without influence from biodegradation. To examine the occurrence of the target compounds in Halifax Harbour, water samples from several locations will be collected, extracted and analysed using a GC-MS and LC-MS procedure for phenolic compounds.

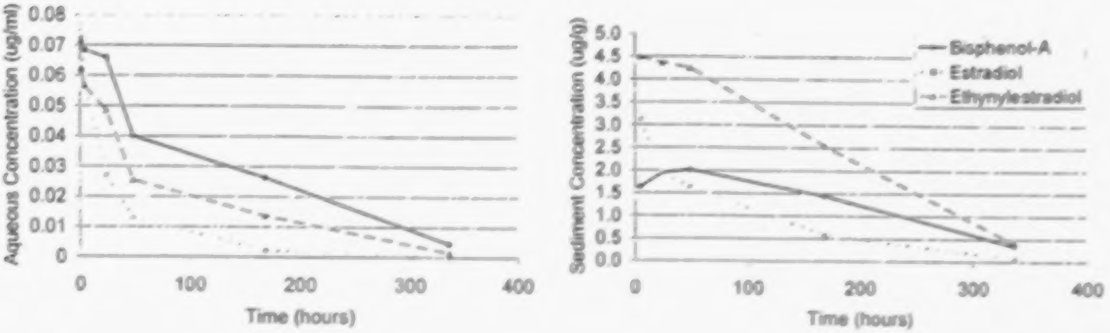

Fig. 3. The change in concentration of bisphenol-a, estradiol and ethynylestradiol in the (a) aqueous phase and (b) sediment phase during the sorption/biodegradation experiment using a Halifax Harbour sample. All three compounds were completely biodegraded after two weeks, although the synthetic compounds were biodegraded more slowly and were found in higher concentrations in the sediment than the natural hormone estradiol.

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Searching for StAR in an endocrine haystack: how is beta-sitosterol affecting cholesterol availability? R.L. Sharpe[1], J. Bonselaar[2], G.J. Van Der Kraak[2], A.J. Woodhouse[3], T.W. Moon[3] and D.L. MacLatchy[1]. [1]Department of Biology, University of New Brunswick, St. John, NB; [2]Department of Zoology, University of Guelph, Guelph, ON; and [3]Department of Biology, University of Ottawa, Ottawa, ON.

A typical consequence of exposure to endocrine disrupting substances in fish is decreased reproductive steroid production and plasma concentration. The first step in steroid biosynthesis is the acquisition of cholesterol from either exogenous sources (i.e. the diet), endogenous stores (circulating lipoproteins) or *de novo* synthesis from acetate. Our lab has shown that exposure to the phytoestrogen β-sitosterol (β-sit) decreases the amount of reactive cholesterol in fish gonadal mitochondria. β-sit is present in a variety of plant species and finds its way into pulp and paper effluent after the processing of wood products. The decreased availability of cholesterol in gonadal tissues following exposure to β-sit suggests cholesterol transfer to the steroidogenic pathway is impeded. Steroidogenic acute regulatory (StAR) protein has been identified as a cholesterol transporter in the inner mitochondrial membrane and is a likely factor involved in cholesterol-mediated endocrine disruption. My research is examining StAR abundance in goldfish that have been exposed to β-sit via Silastic® implants in an effort to determine the specific mechanism(s) by which β-sit decreases steroid synthesis.

Effects of a potent estrogen on a freshwater food web. K.A. Kidd[1], D.L. Finley[2], M.J. Patterson[2], A.G. Salki[2], P.J. Blanchfield[2] and K.H. Mills[2]. [1]Department of Biology, University of New Brunswick, St. John, NB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

We conducted a whole-lake study from 1999-2004 at the Experimental Lakes Area in northwestern Ontario to examine the effects of the potent synthetic estrogen 17*a*-ethynylestradiol (EE2) on a freshwater food web. Lake 260 was continuously dosed with EE2 in each of the summers of 2001-2003 to maintain surface water concentrations between 4-6 ng/L. Water chemistry and microbial, algal, and zooplankton populations were sampled in this lake bi-weekly during the two years prior to dosing and the three years of EE2 additions, and compared to reference lake samples collected over the same time period. In addition, large and small fish species were monitored for population-level effects in the spring and fall of each year in the EE2 amended and reference lakes. Microbial abundances ranged from 0.04-0.3 mg/L in the study lake, and were similar before and after the additions of the estrogen. In contrast, the algal biomass in Lake 260 was lowest in 2003 at 465 mg/m3 compared to 600-900 mg/m3 in previous years, and could be related to increased grazing due to changes in food web structure. Mean annual zooplankton abundances in the study lake were similar from 1999-2002 and ranged between 25-40 individuals/L; in 2003 zooplankton abundance was 2-3 fold higher when compared to previous years' data. This change is likely due to a concurrent decline in minnow abundance observed in this lake. In both 2002 and 2003, year class failures were observed for one of the dominant fish species, the fathead minnow, and the smaller size classes of this species are no longer present in this lake. Results from this study indicate that a potent estrogen can have direct effects on aquatic populations as well as indirect effects through a trophic cascade due to changes in predator and prey abundances.

***In vitro* assessment of biological impacts to rainbow trout by the polycyclic musk HHCB.** D.B.D. Simmons[1], C.D. Metcalfe,[2] G.C. Balch[2], S. O'toole[2], J. Yang[2]. [1]Watershed Ecosystems Graduate Program, Trent University, Peterborough, ON; and [2]Environmental and Resources Studies Program, Trent University, Peterborough, ON.

The polycyclic musk, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyltetralin-(g)2-benzopyran (HHCB), is one of the most widely used man-made fragrances, with European annual usage during

1990's averaging 1473 tonnes. It is an additive in a variety of personal care products ranging from detergents, cosmetics, and household air fresheners. The majority of these products will end up being washed down the drain, and eventually treated by sewage treatment facilities. Unfortunately, HHCB is recalcitrant and removal by sewage treatment plants in Canada is rarely greater than 20%. HHCB can have lifetimes upwards of 10 years in the aquatic environment, and over six months when applied to land as biosolids. HHCB can accumulate in Great Lakes fish in contaminated environments to concentrations approaching 1 mg/kg. Recently, Schruers *et al.* have determined that HHCB is a weak antagonist at the estrogen receptor in zebrafish, both *in vitro* and *in vivo*. This study uses three-tiered approach to confirm these results and to determine biological affects of HHCB in rainbow trout. In the first level, the YES assay was used as a screening method. Results indicated that HHCB was a strong estrogen receptor inhibitor. Currently, level two and three include tests aimed directly at the rainbow trout estrogen receptor. An *in vitro* reporter gene assay in RTG-2 cells contains three plasmids, one with an rtER and the other two containing a dual luciferase reporter gene system. *In vivo* testing in rainbow trout uses a competitive ELISA to quantify the biomarker egg-yolk protein vitellogenin. Preliminary results from these tests support inhibition of the rainbow trout estrogen receptor by HHCB.

Endocrine disrupting effects in fathead minnows exposed to pharmaceuticals and municipal wastewater effluent. J.L. Parrott, B.R. Blunt, C.A. Sullivan and S.M. Rhodes. Environment Canada, National Water Research Institute, Burlington, ON.

Assessing the effects of municipal wastewater effluents (MWWEs) on fish poses a challenge, as the effluents are complex mixtures of nutrients, hormones and pharmaceutical drugs. Lab lifecycle studies of fathead minnows have shown that ethinylestradiol (EE2) is a potent feminizer at 3.5 ng/L, and that lower concentrations (0.35-1.2 ng/L) significantly reduced egg fertilization success and male secondary sex characteristics. Thresholds for effects of EE2 are similar to EE2 concentrations in many North American MWWEs. Masculinization of fish has been seen downstream of pulp and paper mill effluents, and testosterone is commonly detected in MWWEs. The threshold for masculinization of fathead minnows exposed for a lifecycle to methyltestosterone was well below 10 ng/L. Male fathead minnows exposed to 100% model scale MWWE for a lifecycle had increased liver-somatic indices and decreased male secondary sex characteristics (smaller dorsal fatpads and fewer nuptial tubercles). Exposure for a lifecycle to the anti-inflammatory drug indomethacin (360 ng/L measured concentration) reduced gonadosomatic indices of male fathead minnows by 25%. These concentrations of indomethacin are similar to those detected in MWWEs. Assessment of the effects of pure endocrine disrupting substances (estrogens and androgens) allows the development of sensitive and predictive fish tests that will assess the potential reproductive effects of pharmaceutical chemicals and mixed effluents.

Sewage: a perpetual source of pharmaceutical and personal care products in Atlantic coast estuaries. G.L. Brun[1], R. Losier[1], F.L. Comeau[2], H.B. Lee[3], C. Surette[2], P. Falleta[4]. [1]Environment Canada, Environmental Conservation Branch, Moncton, NB; [2]Department of Chemistry and Biochemistry, Université de Moncton, Moncton, NB; [3]Environment Canada, National Water Research Institute, Burlington, ON; and [4]Environment Canada, Wastewater Technology Centre, Burlington, ON.

Untreated and treated sewage are the main sources of pharmaceutical drugs and personal care products in the environment. Several commonly used prescription and over-the-counter drugs were reported previously in sewage treatment plant final effluents and receiving waters of the four Atlantic Provinces. Although wastewater treatment infrastructure is improving, a significant number of communities in the region still do not have adequate sewage treatment. Given that little is known about the effects of many of these biologically active compounds and their metabolites in the environment there is potential for negative impacts in the receiving aquatic environment, especially in large population areas with untreated sewage. In this on-going study three harbours were selected to investigate the occurrence of several drug groups and synthetic musk fragrances in the vicinity of treated and untreated sewage outfalls in Halifax, and Pictou, NS, and St. John's, NL. A total of 21 drugs were selected including some of the more common analgesic and non-steroidal anti-inflammatory (NSAIDs) compounds, lipid regulators, betablockers, antiepileptics, and antineoplastics (cancer treatment chemicals). The samples were also screened for 12 synthetic polycyclic and nitro musk compounds. The interpretation and significance of these results are discussed.

Assessment of immune competency of rainbow trout exposed to municipal sewage effluent. B. Wasserab[1], M.R. van den Heuvel[2], B. Koller[3], B.C. Hitzfeld[4] and D. Dietrich[1]. [1]Konstanz University, Germany; [2]Forest Research Institute Ltd., Rotorua, NZ; [3]German Institute of Viral Diseases of Animals, Germany; and [4]Swiss Agency for the Environment, Switzerland.

The response of rainbow trout to a tertiary treated municipal sewage effluent was examined using a short-term (4 week) exposure with juvenile and a long-term (32 week) exposure with adult rainbow trout. The exposures were conducted concurrently with injections either with inactivated trout pathogen *Aeromonas salmonicida* (A.s.) as an immune system activator, or with buffer alone. Endpoints measured include A.s.-specific antibody production, oxidative burst, phagocytosis, lymphocyte proliferation, lysozyme activity, white blood cell differentials and spleen immunohistopathology. To obtain adequate sample sizes, fish sacrifice was staggered across two days for all experiments/genders. For the short-term exposure, peripheral blood lymphocytes given as percent of total blood cells and lymphocyte proliferation were the only immune variables that responded to effluent exposure. A particularly interesting aspect of this was a dose-response of degrading erythrocytes with exposure concentration, but only on day 2 of sampling, indicating a stress-related effect. The long-term exposure revealed that males and females responded differently to sewage effluent exposure as well as to sampling stress. Spleen immunohistopathology indicated that despite the subtle changes in the functional immune endpoints measured, modification of immune cells in spleen was substantial. Inadvertently, these experiments revealed that low levels of stress could have very significant impacts on immune function as compared to the modest component of variability due to effluent exposure. The stress and exposure effects were also substantially modified by the gender of the trout. Further experimentation in immunotoxicology needs to examine whether responses to chemicals can be attributed to a direct effect on the immune system or are a manifestation of general adaptation.

Effects of pharmaceutical products in aquatic organisms. F. Gagné and C. Blaise. Environnement Canada, Centre Saint-Laurent, Montréal, Qc.

Pharmaceutical, personal care and veterinary products have been found in wastewaters and surface waters and are likely to contaminate the aquatic environment including groundwater. The purpose of this presentation was to examine current and new strategies to evaluate the toxicological effects of this special class of xenobiotics to aquatic species. At the present time, aquatic sentinel species that bioaccumulate some of these drugs are lacking but some studies with mussels and plants showed that significant accumulation in tissues is occurring with some antibiotics.

Laboratory tests have been used with some success with many aquatic species ranging from bacteria, plants, invertebrates (molluscs and arthropods), and fish using single and mixture preparations of commonly found drugs. These toxicity tests generally indicate that acute lethal effects are not likely to happen in the environment but chronic or long-term effects cannot be excluded. In the attempt to measure the effects of pharmaceutical and personal care products, two types of biomarkers are proposed. The first class, defined as integrative biomarkers, consists in measuring ecologically relevant biomarkers that encompass the effects of drugs such as oxidative stress or DNA damage. The second class concerns those that measure the state or integrity of drug targets likely to impede and the organism's health and reproduction. Finally two case studies are presented to exemplify the use of biomarkers to assess the state of drug targets and tissue damage in aquatic species. In the first case study, primary cultures of rainbow trout hepatocytes were used to evaluate the cytotoxicity of carbamazepine® (CBZ), a drug commonly found in municipal wastewaters at mg/L range, after an exposure of 48-h at 18°C.

Results showed that CBZ induced the activity of cytochromes P4503A4 and 2B6 (benzyloxyresorufin as the substrate), known biotransformation enzyme for this drug class (iminostillbene), and was highly correlated with lipid peroxidation and cell viability at environmentally relevant concentrations. Lipid peroxidation and cell viability are considered as integrative biomarkers while cytochrome P4503A4/2B6 activity is a drug target-specific biomarker. The second case study concerns feral carp surviving for 4 years in one aerated lagoon that treats essentially a domestic municipal effluent. Results show that dibenzyloxyfluoresce-indebenzoylase activity (another substrate specific towards cytochromes P450 3A4, 3A5 and 2C9), were readily induced in the post-mitochondrial supernatant of liver homogenates. ATP-dependant dopamine transport activity in synaptosome preparations of brain tissues was shown significantly reduced. Increased cytochrome P450-related activities and reduced dopamine uptake suggest the pharmacological effects of opiate-like substances. Preliminary findings suggest that some aquatic species could accumulate some drugs and these drugs are likely to produce effects at their designed biochemical targets and produce harmful effects. Further research studies are needed to validate these biomarkers and to relate changes in drug target integrity with the levels of drug residues in tissues.

Aquatic toxicity of carbamazepine®, atorvastatin® and triclosan® to benthic invertebrates. È.B. Dussault[1], K.R. Solomon[1], E. Sverko[2] and P.K. Sibley[1]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON; and [2]National Laboratory for Environmental Testing, Environment Canada, Burlington, ON.

Land application of sewage sludge has resulted in the occurrence of pharmaceuticals in aquatic systems. Aquatic sediments may represent an important matrix for the deposition and storage of pharmaceuticals, but the potential risks that these compounds pose to sediment-dwelling organisms is virtually unknown. In this study, we evaluated the acute toxicity of the anti-epileptic drug carbamazepine®, the lipid regulator atorvastatin®, and the antimicrobial triclosan® toward the midge *Chironomus tentans* and the freshwater shrimp *Hyalella azteca*, using standard 10-d waterborne tests. In water–only exposures, LC50 values varied between 0.4 and 34.4 mg/L for *C. tentans*, with triclosan being the most toxic (0.4 mg/L) and carbamazepine the least toxic chemical (34.4 mg/L). *H. azteca* was generally more sensitive than *C. tentans*, with LC50 values ranging from 0.2-17.2 mg/L. The relative toxicity ranking of the three pharmaceuticals was the same as observed for *C. tentans*; however atorvastatin was approximately 10 times more toxic to *H.* azteca compared to *C. tentans*, and approached the toxicity of triclosan. Growth was generally a more sensitive indicator of toxicity than mortality, with EC50 values ranging 0.8-3.8 times lower than LC50 values. It is suspected that the acute toxicity of spiked sediments will be lower than water-only exposures. Measured toxicity thresholds in these short-term tests were several orders of magnitude higher than current environmental concentrations, indicating that these compounds likely pose little risk to benthic invertebrates. However, studies to examine chronic responses to pharmaceuticals, particularly effects on reproduction and behaviour, are poorly known and will be the subject of future testing.

A re-assessment of wild fish from Canadian Areas of Concern for reproductive health. M.E. McMaster, G.R. Tetreault, C. Boyko, S.B. Brown and J.P. Sherry. Environment Canada, National Water Research Institute, Burlington, ON.

Environment Canada has undertaken studies in Canadian Areas of Concern (AOCs) to determine the current state of fish and wildlife reproductive health. Phase One (2001-2005) is focusing on conditions in AOCs of the lower Great Lakes. The studies conducted to date focused on AOCs in western Lake Erie (Wheatley), the Detroit and St. Clair Rivers and Toronto Harbour. Two different fish species (wherever possible) were collected from exposed and reference sites and examined for reproductive health. As part of this study our laboratory measured circulating levels of reproductive sex steroids, the production of these steroid using an *in vitro* gonadal incubation assay as well as gonadal histology for differences in gonadal development due to exposure. These reproductive endpoints were compared to other indicators of overall fish health as well as other endocrine endpoints including thyroid function and circulating vitellogenin levels. This study is ongoing and its overall goal is to reassess all of Canada's AOCs for evidence of endocrine disruption.

Measuring acidic and neutral pharmaceuticals in surface waters by GC-MS and HPLC-MS analysis. F.L. Comeau[1], G.L. Brun[2], C. Surette[1] and R. Losier[2]. [1]Department of Chemistry and Biochemistry, Université de Moncton, Moncton, NB; and [2]Environment Canada, Environmental Conservation Branch, Moncton, NB.

An analytical method was adapted for determining the presence of several acidic (e.g. diclofenac®, ibuprofen®, naproxen®, and salicylic acid) and neutral (e.g. carbamazepine®, acetominophen®) pharmaceuticals in surface waters. Sampling sites in the harbours of Halifax and Pictou, Nova Scotia as well as Saint John's Harbour and a small stream in Gander, Newfoundland were strategically chosen where sewer discharges are located. Following sampling, the surrogates were added to the one litre samples and they were extracted by SPE (solid phase extraction) with an Oasis HLB cartridge before eluting the analytes with ethyl acetate. The acidic compounds were methylated with MNNG (1- methyl-3-nitro-1 nitrosoguanidine) before GC-MS analysis, and the neutral compounds were analysed directly by HPLC-MS. This method proved efficient, enabling detectable concentrations of these compounds at the low ng/L level.

Effects of testosterone and 5α-dihydrotestosterone on plasma IGF-1 and growth of Atlantic salmon smolts. J.T. Arsenault[1], W.L. Fairchild[1], D.L. MacLatchy[2], K. Haya[3], L.E. Burridge[3] and S.B. Brown[4]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Department of Biology, University of New Brunswick, Saint John, NB; [3]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB; and [4]Environment Canada, National Water Research Institute, Burlington, ON.

Previous studies have shown that short-term, water-born exposures of Atlantic salmon (*Salmo salar*) smolts to environmental levels of estrogen and 4-nonylphenol affect parr-smolt transformation (PST) such that subsequent growth in seawater was impaired and plasma Insulin-like Growth Factor-1 (IGF-1) concentrations were reduced. IGF-1 is one of the key hormones that modulate PST and growth. The present study was conducted to determine the effects of low-level, water-borne exposures of an aromatizable androgen [i.e. Testosterone (T)] and of a non-aromatizable androgen [i.e. 5α-Dihydrotestosterone (5α-DHT)] on smolt growth and plasma IGF-I concentrations. Smolts were exposed to environmentally-relevant sustained water-borne doses of either T (10, 100, 1000 ng/L) or 5α-DHT (100, 1000 ng/L) for six days. Treatments occurred in mid-May in fresh water, during the final stages of PST. Subsequent smolt growth in seawater and plasma IGF-1 concentrations were evaluated. Preliminary results show that both T and 5α-DHT negatively effect smolt growth and plasma IGF-I concentrations, and that their effects are similar. Therefore, testosterone is likely acting via an androgenic mechanism. These results are significant; androgenic compounds stemming from various anthropogenic activities could influence fish populations.

Toxicological evaluation of Georgia Basin municipal waste water effluents (MWWE). J. Bruno and G.C van Aggelen. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Traditional toxicological endpoint measurements fail to adequately measure the effects of low level contaminants. New predictive tools using toxicogenomics, a field of toxicology utilizing molecular techniques to determine gene expression alterations due to material exposures, are gaining support for their ability to measure molecular level toxicological effects. This is particularly relevant to the new generation of environmental toxicants commonly referred to as endocrine disruptors. Environment Canada's Pacific Environmental Science Centre (PESC) toxicology section with its expertise in genomics and gene array technology developed a salmonid cDNA microarray incorporating over 200 gene transcripts. Collaborators at the University of Victoria (Helbing laboratory) produced an amphibian microarray with over 450 gene transcripts. These unique species-specific microarrays are used to examine gene expression changes in organisms exposed to a variety of contaminants. One toxicogenomic evaluation is the Georgia Basin Action Plan (GBAP) MWWE studies. As part of the five-year collaborative programming in the Georgia Basin of British Columbia, PESC's study objectives are to: (1) evaluate the occurrence and concentrations of selected pharmaceutical and personal health care products (PPCP) in MWWE, and (2) produce toxicogenomic expression profiles for salient indicator species. Freshwater and marine water bioassays are being conducted with tissues harvested for molecular work-up. Gene expression profiles provide an integrative view of the organisms' response to the chemicals as a mixture.

Targeted proteomics to measure vitellogenin in rainbow trout. M. Smith[1], M.R. van den Heuvel[1] and N. Ling[2]. [1]Forest Research Institute Ltd., Rotorua, New Zealand; and [2]Centre for Biodiversity and Ecological Research, Waikato University, Hamilton, New Zealand.

Vitellogenin (Vtg) is a large phospholipoglycoprotein and a precursor of the major egg-yolk proteins that are sources of nutrients to offspring during early development. Although vitellogenesis is a highly conserved biological process among oviparous animals, it is not a well-conserved protein. Because estrogens hormonally regulate Vtg expression, the protein has been used in toxicological studies as an indicator of exposure of fishes to estrogenic substances. Typically, enzyme-linked immunosorbent assay (ELISA) and Vtg mRNA methods have been used for Vtg quantitation. These methods, however, suffer from drawbacks such as antibody specificity and the need to harvest fresh liver tissue. The recent emergence of the field of proteomics has enabled rapid and sensitive methods for protein identification and quantitation. Proteomic methodologies utilise site-specific proteases, liquid chromatography, mass spectrometry and bioinformatics tools to measure protein expression. In this study, a proteomics method was developed in order to quantify plasma Vtg levels of rainbow trout (*Oncorhynchus mykiss*) exposed to sewage treatment works (STW) effluent. The results were compared to values obtained with an ELISA. The calculated concentrations (ng/ml) of plasma Vtg obtained using the proteomic method were shown to correlate well with ELISA results. The proteomic method was shown to be a more sensitive indicator Vtg gene expression than the ELISA while to be utilized for the measurement of proteins for which alternative methods do not exist, or are difficult to acquire, will be discussed.

Artifactual toxicity in municipal waste water - ammonia and pH / Toxicité artficielle des eaux usées municipales - ammoniaque et pH

Session co-chairs: G.C. van Aggelen and J.D. Clarke

Municipal experiences with apparent effluent ammonia toxicity. A. van Roodselaar, R. Ng, B. Hystad, G. Marsh and S. Bertold. Greater Vancouver Regional District, Burnaby, BC.

The Greater Vancouver Regional District (GVRD) operates five wastewater treatment plants. The effluent from these plants contains variant concentrations of ammonia. At monthly intervals, samples from these plants are taken and sent to laboratories certified to carry out 96-h LC50 fish bioassays. The GVRD has experienced LC50 values of less than 100% using the standard Environment Canada test with secondary treatment plant effluent. Early GVRD trials in which aeration was not carried out during the test procedure showed that these samples would pass the LC50 test, when parallel samples that were subjected to aeration during the test procedure would not. A further set of trials was undertaken in which the fish were tested in an actual side stream of effluent taken from the plant and flowed through the test cells. Over a six month period, monthly 96-h LC50 tests were undertaken in this test system and were successful even when concurrent standard tests were not successful. Currently the GVRD uses a procedure developed by Environment Canada's Pacific Environmental Science Centre Laboratories in parallel with the standard test procedures when testing treatment plant effluent. To test the concern that the effluent might become toxic in combination with river water, the GVRD undertook a set of serial dilutions. This was to determine if at any point during the dispersion process an effluent/river water mixture might have a combination of pH and ammonia concentration conditions that might prove to be toxic. This question was driven by the fact that the river water was at a higher pH than the effluent.

Criteria and supporting rationale for applying to use the pH stabilization procedure during the testing of acute lethality of municipal wastewater to rainbow trout. R.P. Scroggins[1], L.J. Novak[2] and K.E. Holtze[2]. [1]Environment Canada, Environmental Technology Centre, Ottawa, ON; and [2]Stantec Consulting, Guelph, ON.

In many municipal wastewater effluents, the CO2 content may be artificially elevated due to the presence of high biological activity, or a result of wastewater acidification prior to discharge. Consequently, aeration of a CO2 saturated wastewater sample (such as during the Environment Canada rainbow trout acute lethality test) may cause pH to rise due to the equilibration of CO2 partial pressure in the wastewater with that in the atmosphere. Any change in wastewater pH during a toxicity test may affect acute lethality if the substance responsible is pH dependent. Ammonia, which is of particular concern in municipal wastewater effluents, would be one such example of a pH dependent toxicant. Depending on the initial pH of the full-strength wastewater and the magnitude of the pH shift during testing, concentrations of un-ionized ammonia that were below lethal levels at test initiation, could increase sufficiently during testing causing rainbow trout mortality by test completion. This is termed "artifactual" toxicity, as the observed mortality may be the result of the test conditions (i.e., pH increase caused by aeration). The purpose of this presentation will be to outline the specific criteria and supporting lethality tests with municipal wastewater effluents to account for artifactual toxicity caused by the presence of ammonia and shifts in pH during test aeration.

pH Control by carbon dioxide addition for Environment Canada's rainbow trout acute lethality test: a review and update of method development. G.C. van Aggelen and G.R. Schroeder. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

The Pacific Environmental Science Centre (PESC) has conducted research for the past three years into the issue of pH drift induced artifactual ammonia toxicity in Environment Canada's rainbow trout acute lethality test. The results of testing with over 60 samples of municipal waste water effluent (MWWE) have provided sufficient evidence to conclude that upward pH drift during testing may cause artificially induced ammonia toxicity. The concern over the validity of results from compliance tests under these conditions has led PESC to develop a method to control pH. The method involves the addition of carbon dioxide to prevent the upward pH drift that normally occurs under standard aeration conditions. The method has been developed to ensure that it is a practical, flexible, cost-effective, and precise method to control pH while retaining the integrity of the rainbow trout acute lethality test. The method has undergone several stages of development and peer review within Environment Canada and the Ontario Ministry of Environment. Results from testing with MWWE samples have provided evidence that the method is able to effectively eliminate pH drift induced artifactual ammonia toxicity. The method is ready to be adopted by Environment Canada as a standard method to control pH for the rainbow trout acute lethality test. The results demonstrating the ability of carbon dioxide addition method to control pH in tests with municipal waste water effluents (MWWE) samples will be presented. The current technical status of the pH control method will also be presented.

Inter-laboratory toxicity evaluation of pH stabilization procedures for use with municipal wastewater. L.J. Novak. Stantec Consulting Ltd., Guelph, ON.

An inter-laboratory study was conducted to evaluate two procedures for pH stabilization during the 96-h rainbow trout acute lethality test: (1) CO2 recycling in a contained headspace, and (2) direct CO2 addition. Prior to the round-robin study, pre-screening was conducted using effluent from three municipal wastewater treatment plants. Testing confirmed that: (1) samples (as received) were non-acutely lethal to rainbow trout, (2) effluent pH drift (> 0.2 pH units) was observed during testing, (3) significant ammonia, (4) the un-ionized ammonia concentration at test initiation was < 1.0 mg/L, and (5) artifactual toxicity could be eliminated by one or both pH stabilization procedures. Based on these results, an effluent source was selected for the round-robin study. The purpose of the study was to: (1) assess the performance of the two pH stabilization procedures, (2) estimate between-laboratory variability of the two procedures, and (3) demonstrate the effectiveness of the two procedures in controlling artifactual toxicity. Five government laboratories participated: (1) Pacific Environmental Sciences Centre, Vancouver, (2) Prairie and Northern Region, Edmonton, (3) Ontario Ministry of the Environment, Toronto, (4) Centre St. Laurent, Montreal, and (5) Environmental Science Centre, Moncton. Initial results suggest both methods can be successfully applied using either single or multiple concentration test methods.

Toxicological assessment of ammonia using Environment Canada's rainbow trout acute lethality test (RM9). G.R. Schroeder and G.C. van Aggelen. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Ammonia is widely known to be a substance that is potentially deleterious to aquatic life and is listed as a toxic substance in Schedule 1 of CEPA 1999. However, the historical ammonia acute toxicity toxicological database is obscure for rainbow trout. There is confusion about the toxicity of the total ammonia and its un-ionized species in the historical database. The historical ammonia toxicity data based on the current Environment Canada standard protocol of the rainbow trout acute lethality test (RM9) is sparse. The relationship between pH and ammonia toxicity to rainbow trout has not been sufficiently researched and understood. The Pacific Environmental Science Centre (PESC) has evaluated the toxicity of total and un-ionized ammonia across a pH range of 7.0-9.75 and three water hardness types using the rainbow trout acute lethality test. The results have quantified the relationship between ammonia toxicity and pH for the rainbow trout. The results provide clear evidence that the toxicity of total ammonia to Rainbow trout increases as pH increases. The research also indicates that un-ionized ammonia becomes less acutely toxic as pH increases. The ammonia toxicity results from the pure ammonia tests also correspond well with the results of tests conducted on "real world" municipal waste water effluent samples. The data provided by ammonia toxicity testing with the rainbow trout will allow scientists, engineers and waste water treatment plant operators to effectively assess the potential toxicological impact of the release of ammonia into the Canadian aquatic environment.

Site-specific surface water quality objective for ammonia plus chloramine. G.R. Craig[1], I. Middelraad[2] and D.G. Dixon[3]. [1]G.R. Craig & Associates, Schomberg, ON; [2]I. Middelraad & Associates, Guelph, ON; and [3]D.G. Dixon & Associates, Guelph, ON.

Ecological risk associated with treated municipal wastewater as a result of separate exposure to ammonia and chloramine has been documented in national assessments by Environment Canada. This objective was developed as part of an Environment Canada risk management strategy for joint exposure to ammonia and inorganic chloramines. The aquatic life threshold effect concentrations for both ammonia (0.041 mgNH3/L) and chloramine (0.010 mg chloramine/L) were adapted from the respective Environment Canada assessments to develop a joint toxicity application based on strict addition. Site-specific chloramine decay is determined empirically and incorporated into the dispersion/dilution model to identify a three dimensional exposure area where the joint objective would be exceeded. When exposure above the objective overlaps areas of sensitive use, management of effluent quality or dispersion would be required to avoid conflicts. Justification is provided for toxicant addition and the conservative nature of the threshold effect concentrations selected. Numerical and graphical examples of the application of the joint toxicity objective are provided.

pH Controller technology and it's application to pH stabilization in rainbow trout acute lethality tests. R. Chong-kit and J.E. Schroeder. Ontario Ministry of Environment, Etobicoke, ON.

The Ontario Ministry of the Environment (MOE) participated in a round robin testing program to evaluate two methods for stabilizing pH in exposures of municipal effluent. The methodologies, one using CO2 injection and the other using headspace air recycling, were developed by Environment Canada to stabilize sample pH during a 96 h exposure of rainbow trout, tested according to the Environment Canada standard method (EPS 1/RM13). In addition to the evaluation of the two Environment Canada techniques, the MOE adapted pH controller technology and evaluated its application to pH stabilization in the acute lethality test. The pH controller technique built on the CO2 injection approach and improved it by reducing the cost of the test system and simplifying setup and maintenance while equalling the pH control performance. A description of the pH controller technique will be presented along with a comparison to the two Environment Canada test systems in terms of materials cost, setup and maintenance, and pH control performance.

Changes in metal bioavailability and speciation along a municipal wastewater effluent dispersion plume. C. Gagnon[1], P. Turcotte[1] and B. Vigneault[2]. [1]Environnement Canada, Centre Saint-Laurent, Montréal, Qc; and [2]Natural Resources Canada, CANMET Mining and Mineral Sciences Laboratories, Ottawa, ON.

Environmental impacts of urban wastewater discharge on receiving waters are numerous and discharged contaminants such as metals may be incorporated in the biota. The biological availability and speciation of metals released from municipal wastewater effluents is strongly influenced by the physico-chemical conditions of the receiving waters. Results from a previous study on metal bioaccumulation in caged mussels showed that metals are generally less bioavailable in the effluent dispersion plume than at the reference site in the Saint-Lawrence river. The objective of this study was to determine the changes in physical and chemical forms of metals in the receiving waters of a major urban effluent. The effluent dispersion plume generated by the wastewater (Québec), which is the largest one in the St. Lawrence Valley, was investigated under this study. Surface water and suspended particulate matter were sampled with using in situ dialysis and sediment traps at several sites, 0.5-15 km downstream of a municipal outfall plume in the river. Total and extractable particulate as well as total dissolved, colloidal, and free metal concentrations were determined in surface waters with a range of speciation techniques: filtration, ultrafiltration, ion exchange and equilibrium model calculations. Partitioning of metals between dissolved and particulate phases varies along the effluent dispersion plume and therefore could strongly influence the exposure routes for aquatic organisms. Results of both chemical and physical speciation explain the low metal bioaccumulation in gills of mussels exposed to the effluent. Environmental factors such as organic carbon concentration and the presence of colloids appear to control the environmental fate of metals in the receiving waters.

Development of a methodology for testing the toxicity of ammonia to aquatic invertebrates at low temperature and low pH. D.G. Poirier[1], J. Van Geest[2] and A. Tomczyk[2]. [1]Ontario Ministry of the Environment, Laboratory Services Branch, Etobicoke, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

Our understanding of ammonia speciation and toxicity at various pH's is based on a limited range of temperature data. It is recognized that, generally, the NH3 component is most toxic of the NH3/NH4+ complex to aquatic organisms, but some concern has been expressed that the toxicity of the NH4+ component of the complex may be underestimated at low pH and low temperature. When provincial and federal water quality objectives were developed for ammonia, there was a distinct gap in the toxicity data at temperatures between 5-15°C and pH's between 6.0-7.0. This is of particular interest to mining operations and municipal waste water treatment plants operating in northern communities, where receiving waters of low pH and low temperature are the norm, and ammonia may be the primary toxicant of concern in their discharges. This study looked at the development of a methodology to measure the toxicity of NH3/NH4+ to *Daphnia magna* at low temperatures. Starting with a well established test method ("Biological Test Method: reference method for determining acute lethality of effluents to *D. magna*" (Environment Canada EPS 1/RM/14)) and make minor adjustments to allow us to measure toxicity at low pH and temperature. This method development looked at: (1) acclimation requirements for using *D. magna* at low temperatures, and (2) pH adjustment/stabilization strategies to maintain pHs of 6.0, 6.5, and 7.0. Preliminary data indicates that there is either an increased sensitivity of *D. magna* to ammonia at low temperatures (physiological or acclimation changes) or an increase in toxicity of ammonia complex under these conditions.

Sediment and Soil Toxicity / Toxicité des sédiments et des sols

Session co-chairs: K.G. Doe and R.P. Scroggins

Validation of Environment Canada biological test methods for assessing contaminated soils: earthworm and plant toxicity tests. J.I. Princz and R.P. Scroggins. Environment Canada, Environmental Technology Centre, Ottawa, ON.

Environment Canada is continuing its efforts to develop, validate, and publish standardized toxicology methods for the testing of field-contaminated and substance-amended soils. Standardized toxicity test methods have been developed using species representative of terrestrial invertebrates (e.g., earthworms and arthropods) and plants inhabiting soil ecosystems in Canada. Prior to publication, each soil toxicity method must be peer reviewed by experts and validated through inter-laboratory testing. Environment Canada has co-ordinated a series of inter-laboratory studies involving 12 laboratories across Canada. The first series of round-robin studies focused on assessing the various earthworm toxicity test options using the species, *Eisenia andrei*. The earthworm studies included an assessment of the 7-day reference toxicant test using artificial soil amended with boric acid, as well as the 14-day lethality and the 48-h acute avoidance tests using a field-collected reference soil amended with boric acid. The second series of round-robin studies will focus on two plant tests, the 7-day reference toxicant using artificial soil amended with boric acid, and a longer-term test involving amended and field-contaminated soil. The mean 7-day LC50 for earthworms was 3,826 mg H3BO3/kg soil dry wt., with values for individual laboratories ranging from 3,236-4,198 mg/kg. The co-efficient of variation was 9%, indicative of good laboratory precision. The mean 14-day LC50 for earthworms was 3,524 mg/kg, with values for individual laboratories ranging from 2,228-4,677 mg/kg. The coefficient of variation was 25%, an acceptable level of between-laboratory precision. The results obtained from each of the earthworm and plant test options will be summarized and discussed with regards to the observed within and between-laboratory variability.

***Eisenia fetida* or *E. andrei*: which species are you using?** J.H. McCann and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Earthworm test methods generally recommend the use of the earthworm species *Eisenia fetida* and/or *Eisenia andrei*. There is some confusion regarding the taxonomic classification of these two species. Morphologically, they are identical and cannot be positively distinguished by external features and, in the past, they were considered to be the same species (referred to as *E. fetida*) or two subspecies of the same species (i.e., *E. fetida fetida* and *E. fetida andrei*). Evidence has accumulated supporting the classification of these earthworms as two distinct species. Since these two species cannot be distinguished based on morphological features, other methods must be employed. One method is the use of horizontal starch gel electrophoresis to assay for allozymes specific for each species. This technique will be introduced and discussed in reference to its use in earthworm identification.

Use of an avoidance test with earthworms for screening reference soils and soils contaminated with petroleum hydrocarbons. N.C. Feisthauer, D.L. Holtze, J.T. Crumb and G.L. Stephenson. Stantec Consulting Ltd., Guelph, ON.

The standard approach to assessing the toxicity of contaminated soil usually consists of using short- or long-term toxicity tests. Environment Canada has recently published a biological test method for earthworms that describes an acute lethality and chronic reproduction test. This new test method also includes an acute sublethal screening test that assesses avoidance behaviour of earthworms to contaminated soil. Research done to date suggests that the 48-h earthworm behaviour test is as sensitive to contaminated soil as a 56- or 63-day chronic test. However few data exist that can be used to correlate the sensitivity of earthworm avoidance behaviour to reproduction when exposed to contaminated site soils. To this end, site soils with PHC contamination were collected and evaluated with the avoidance behaviour test using the earthworm, *Eisenia andrei*. The soils originated in Alberta and had undergone different degrees of remediation to reduce the petroleum hydrocarbon concentrations to stable residuals in soil. Environment Canada's new avoidance behaviour test was compared to the more traditional approaches. The results of avoidance tests with 8 site soils, two reference soils, and a negative control soil were compared to those of the acute (14-day survival) and chronic (35-day survival, 63-day reproduction and growth) screening tests to determine the "sensitivity" of the different tests and to test the hypothesis that the avoidance test is equally, or more, sensitive than the other test methods. The discussion of the comparative results will place this test in perspective in terms of its value as a screening tool in site soil and risk assessments.

Influence of ammonia, pH, dissolved organic carbon and other potentially confounding factors on sea urchin porewater toxicity tests. R.S. Carr[1], M. Nipper[2] and J.M. Biedenbach[1]. [1]United States Geological Service, Marine Ecotoxicological Research Station, Corpus Christi, TX; and [2]Texas A&M University, Center for Coastal Studies, Corpus Christi, TX.

Porewater toxicity tests have been routinely employed to assess the bioavailability of sediment-associated contaminants since the late 1980s. One of the concerns with this testing approach that has contributed to the reluctance of regulators to adopt porewater testing is the potential influence of confounding factors such as pH, ammonia, sulfides and dissolved organic carbon (DOC) on the interpretation of the results. Since the early 1990s, numerous large-scale comprehensive sediment quality assessment studies have been conducted in coastal areas of the US and elsewhere using identical testing protocols. This database is comprised of porewater toxicity tests with sea urchins that have all been conducted in our laboratory using identical protocols for more than forty different studies. This database affords an opportunity to examine the influence of these potential confounding factors on the test results. In addition, some specific experiments for various parameters were conducted within a range of concentrations that have been observed in field collected samples to specifically evaluate the effects of these parameters on the different test endpoints. The results of the database analyses and these additional experiments will be presented to aid in the interpretation of porewater toxicity test results from past and for future studies.

Research needs to improve echinoid fertilisation and development bioassays in Environment Canada's *Disposal at Sea Regulations*. S. Agius. Environment Canada, Environmental Protection Service, Gatineau, QC.

The Disposal at Sea Program of Environment Canada is investigating the possibility of combining an echinoid larval development assay with a revised echinoid fertilization test method, to provide a new bioassay in its battery of sediment regulatory tests. In preparation for this endeavour, a protocol review was conducted to identify test methods that are generally agreed upon, versus areas where essential research is needed before a standard test protocol can be developed. Some key areas for further research include: (1) the use of particular species as "benchmarks" of sensitivity, (2) alternative fertilization endpoints, (3) the overall method for a larval development assay, including alternatives to the "normal" embryo score, (4) sample salinity adjustment, (5) the role of ammonia in regulatory decision making, (6) potential artifacts that result from embryo and larval storage, (7) the adequacy of Canadian reference sites, and (8) the merits and pitfalls of using porewater, elutriate, or bedded sediment in sediment regulatory testing.

Exposure of sphaerid clams to aquaculture waste in an ***in situ*** **sediment bioassay.** M. Kullman[1], K.A. Kidd[1] and C. Podenski[2]. [1]Department of Biology, University of New Brunswick, Saint John, NB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Increasing concerns related to freshwater aquaculture have encouraged research into describing the potential environmental impacts of this industry. A whole ecosystem study at the Experimental Lakes Area in northwestern Ontario has been conducted to assess the impacts of cage aquaculture. In consultation with aquaculture farmers, 10,000 rainbow trout were farmed in 2003 and 2004. For this study, the spatial extent and magnitude of the impacts of aquacultural waste on the benthic community were assessed through a sediment bioassay of the fingernail clam *Sphaerium simile*. Survival, growth, reproduction, and metal body burdens were evaluated for individual clams exposed for 6 weeks to sediment collected from directly below, and from 1, 3, 5, 8, and 50 m in a transect away from the fish cage. The clams exposed to sediment from directly under the cage suffered 100% mortality likely because of the lower density of the sediments (0.09 g ww/cm^3) as a result of fish waste accumulation at this site, compared to sites further from the cage (0.11-0.12 g ww/cm^3). Directly under the cage, copper and zinc levels were five times greater than all other sites, at sediment concentrations of 109 mg/kg and 1054 mg/kg respectively. Cu is below the probable effect level (PEL) in freshwater sediment of 197 mg/kg recommended by the Canadian Environmental Quality Guidelines (CEQG), however, the Zn concentrations in the sediment directly under the cage are five times greater than all other sites, and three times greater than the PEL of 315 mg/kg as suggested by CEQG. Clams at all other sites had zero mortality and low tissue concentrations of Cu (3.9-5.4 mg/kg) and Zn (23.9-32.3 mg/kg), consistent with the low concentrations of copper and zinc observed in the sediment (14.2-22.2 mg/kg and 116-152 mg/kg respectively). Clams exposed to sediment from 1 m away from the cage showed significantly greater growth ($p<0.05$) than clams exposed to all other sediments. Reproductive data suggests increasing average embryo size and number of embryos per adult with increasing proximity to the cage. No differences in sediment quality were found to explain the greater growth or reproduction of clams in sediments from near the cage; C/N ratios and percent carbon were consistent in sediments collected 1, 3, 5, 8 and 50 m from the cage. Results from this study suggest that the effects of rainbow trout aquaculture on fingernail clams mainly occur directly under the cage or within a close radius. This sediment bioassay is an effective method for testing the impacts of cage culture on the growth, reproduction and survival of benthic invertebrates such as the fingernail clam.

Evaluating a suite of sediment toxicity tests to aid in the development of sediment quality guidelines for use by the Disposal at Sea program. D. Lee[1], K.G. Doe[2], G.C. van Agglen[3], C. Buday[3], L. Meloche[4] and C. Wong[1]. [1]Environment Canada, Environmental Protection Branch, Vancouver, BC; [2]Environment Canada, Environmental Science Centre, Moncton, NB; [3]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC; and [4]Resource and Environmental Management, Simon Fraiser University, Burnaby, BC.

A series of sediment toxicity tests were performed on polychlorinated biphenyl (PCB) spiked sediments. The toxicity tests chosen include the current toxicity tests utilized in Environment Canada's Disposal at Sea waste assessment framework, namely: (1) a 10-day acute lethality test using the marine/estuarine amphipod, *Eohaustorius estuarius*; (2) Microtox® solid-phase toxicity test; (3) a 28-day *Macoma balthica* bedded sediment (bioaccumulation) test; and (4) a 14-day polychaete growth and survival test using *Polydora cornuta*. Additional procedures currently under development were also used. These include: (1) an echinoid development test using *Dendraster excentricus*; and (2) a 28-day exposure of ethylene vinyl acetate (EVA) film to PCB spiked sediment to determine approximate chemical bioavailability in sediment. The objectives of this study are to: (1) evaluate the relevancy of the current suite of biological tests employed by the Disposal at Sea Program; and (2) conduct standardized sediment toxicity tests employed by the Disposal at Sea program and additional tests, as outlined in the CCME Spiked-Sediment Toxicity Test (SSTT) Approach in support of finalizing interim PBC sediment quality guidelines. The finalization of interim sediment quality guidelines and/or the development of new sediment quality guidelines for relevant contaminants of concern, where no guidelines exist has been identified as a priority issue by both Environment Canada's National Disposal at Sea program, and the Regional Guidelines Advisory Group, as well as by various provincial agencies.

Assessing the toxicity of refrigerated and frozen stream sediments using Japanese medaka embryo-larval bioassays. C.F. Jardine and K.L. Teather. Department of Biology, University of Prince Edward Island, Charlottetown, PE.

Japanese medaka embryo-larval bioassays were used to assess the toxicity of refrigerated and frozen stream sediment from a region of Prince Edward Island highly impacted by potato farming. Three test sites were chosen on the Wilmot River (W1, W2, W3) and one control site, from a region of low agricultural impact, was chosen on the West River (C). Sediment samples were collected from each of the four rivers in March, 2004. Sediment from each site was thoroughly mixed and placed into 1000 ml jars. About half the sediment was stored at 4°C for approximately two weeks while the other half was frozen for approximately 8 weeks. The toxicity of both cold and frozen stored whole sediments were assessed using embryo-larval bioassays. Newly fertilized medaka eggs were exposed to whole sediments for no longer than 28 days. Endpoints included survivorship, time to hatch, length at hatch, and developmental abnormalities. There were no significant differences between the lengths, survivorship, developmental abnormalities, and hatching times of medaka exposed to refrigerated sediments from W1, W2, W3 or the control region. In addition, there were no significant differences in any of these endpoints for medaka exposed to frozen sediments from W1, W2, W3 or C. However, the lengths of medaka exposed to frozen W1, W2, W3, and frozen West River sediment were all significantly less then the lengths of medaka exposed to refrigerated sediments from those sites. There were also significantly more developmental abnormalities in medaka exposed to frozen sediments than those exposed to refrigerated sediment. Finally, the was some evidence that hatching times for medaka exposed to frozen sediment were shorter than for medaka exposed to refrigerated sediment.

Evaluating the ecological relevance of sediment quality guidelines. C. Wong. Environment Canada, Environmental Protection Branch, Vancouver, BC.

The objective of this study is to evaluate the ecological relevance of sediment quality guidelines. *National Interim Sediment Quality Guidelines* and British Columbia standards for contaminated sites are compared with synoptic chemistry, taxonomy and toxicity data collected from False Creek, Vancouver, BC. Six sampling sites were selected for a similar particle size profile and a range of chemical concentrations. Chemical analyses measured total, total recoverable and dissolved trace metal concentrations, polyaromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, organochlorine pesticides and dioxins and furans. Benthic organisms retained on a 1 mm and 0.3 mm sieve were identified to the lowest possible taxonomic level. Toxicity tests included solid and liquid phase Microtox®, 28-day survival and growth of *Leptocheirus plumulosus* in bulk sediment and fertilization and development of *Dendraster excentricus* in pore water. Physical characteristics of sediment that influence the toxicity of associated contaminants, such as particle size, total organic carbon, acid volatile sulphide to simultaneously extracted metals ratio, ammonia and redox potential were also measured.

Small bodied fishes - application to environmental monitoring / Les poissons de petite taille - utilisation dans les suivis environnementaux

Session co-chairs: K. Gromley and B.J. Galloway

Toxicity and EROD-inducing potency of alkylated polycyclic aromatic hydrocarbons (PAHs) in fish. D. Turcotte[1], M. Bowerman[2], P.V. Hodson[2] and R.S. Brown[1]. [1]Department of Chemistry, Queen's University, Kingston, ON; and [2]Department of Biology, Queen's University, Kingston, ON.

Previous embryo toxicity tests with Japanese medaka (*Oryzias latipes*) have indicated higher toxicity of C1-C4 alkyl-phenanthrenes (alkyl-PHE) and alkyl-anthracenes (alkyl-ANT) than the non-alkylated PHE and ANT compounds. The general toxicity of PHE and ANT is known to be enhanced by activation processes, including activation by cytochrome oxidases (CYP) from postmitochondrial supernatant fraction (S9 fraction) and photoactivation by light. Initial experiments in our group have suggested a link between intrinsic activation of C1-C4-PHE by Cytochrome oxidases and the enhanced toxicity of the alkylated compounds. In this work, we are investigating the S9 and photoactivation of alkyl-PHE and alkyl-ANT to compare the effects on toxicity with activation of PHE and ANT. Toxicity will be indicated by embryo toxicity of the medaka and by EROD induction in juvenile trout (*Oncorhynchus mykiss*). Current experiments show that S9 activation of alkyl-anthracenes produces different derivatives than photoactivation. The photo-activated and non-activated compounds do not show the same level of toxicity in medaka. A comparison of EROD activity for the alkyl-ANT and their photoactivated derivatives has been done with juvenile rainbow trout. These results show that photoactivation of certain non-inducing PAHs (like anthracene) can yield derivatives that induce EROD activity in fish. Future work will include an investigation of the ability of activated alkyl-PHE to induce EROD activity in rainbow trout, including quantitation at the protein level with immunoblotting of the induced CYP.

Measuring multixenobiotic resistance *in vivo* in fish. S.M. Bard[1] and J.J. Stegeman[2]. [1]Faculty of Science, Dalhousie University, Halifax NS; and [2]Woods Hole Oceanographic Institution, Woods Hole, MA.

P-glycoproteins (P-gps) confer multidrug resistance in tumor cell lines; whether P-gps provide in vivo resistance against accumulation of xenobiotics in natural populations of vertebrates is the focus of this study. We have previously observed elevated hepatic P-gp levels in fish exposed to contaminants both in the laboratory and at polluted field sites. The purpose of this study was to characterize how P-gp affects the intracellular disposition and retention of a xenobiotic *in vivo*. We developed an *in vivo* assay to evaluate P-gp-mediated transport of a model substrate, rhodamine B (rhB), in multiple organs of killifish (*Fundulus heteroclitus*). *In vivo* inhibition of P-gps by the chemosensitizer cyclosporin A significantly decreased biliary efflux of rhB (84% lower than in rhB only fish), decreased accumulation in liver (29% lower) and gut (26% lower), and increased accumulation in blood (49% greater than in rhB only fish), brain (225% greater), and ovary (226% greater). No significant differences in rhB accumulation were observed in gill or kidney. Our results indicate that P-gps play a major role in transport of xenobiotics in fish, especially in liver, brain, and ovary. We used this assay to evaluate whether P-gp is involved in the transport of benzo[*a*]pyrene (B[*a*]P), a common marine pollutant which is known to induce P-gp *in vitro*. Our results indicate that the distribution of B[*a*]P and/or its phase I metabolites in liver, brain,and ovary are not influenced by P-gp. We conclude that B[*a*]P should not be considered a P-gp substrate in killifish and likely other vertebrates.

A search for the cause of reproductive steroid depressions in fish exposed to pulp mill effluents. K.S. Shaughnessy[1], A.M. Belknap[2], L.M. Hewitt[3] and D.L. MacLatchy[1]. [1]Department of Biology, University of New Burnswick, St. John, NB; [2]Department of Environmental Biology, University of Guelph, Guelph, ON; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

Since 1997, a number of approaches (artificial stream exposures, lab bioassays) have been used to identify waste-stream sources of contaminants at the Irving Pulp & Paper mill in Saint John, NB. These studies have shown that chemical recovery condensates have the greatest potential for reducing circulating and gonadal steroids in mummichog (*Fundulus heteroclitus*), an endemic fish species. A solid phase extraction technique was developed to isolate the hormonally–active substances from the condensates, and a toxicity identification evaluation (TIE) approach was used to gain a better understanding of the chemical characteristics of the HAS. The extract was fractionated by high performance liquid chromatography and the fractions were used in a 7-day bioassay. Mummichog were exposed in static aquaria with daily renewal to either the whole condensate extract, or one of the six fractions at 1% v/v. Preliminary results that a specific fraction containing naphthalene and molecular sulfur had the greatest potential to reduce plasma testosterone, however, the steroid reductions were not as pronounced as had been observed during studies which investigated the overall potency of the extract at the same concentration. Therefore, further investigations focused on determining whether the condensates had changed in hormonal potency, and whether the extract is an accurate representation of the whole condensates. A dose-response experiment indicated that greater steroid reductions are elicited at 4% v/v in male mummichog. Therefore the TIE was continued at 4% v/v, however, no steroid reductions were observed in any of the fractions. Some fractions induced increases in plasma testosterone, which had never been observed previously. Ongoing work is focusing on developing an understanding of the nature of variability within the condensates, and if this variability can be linked to the inconsistencies observed within the 7 d mummichog bioassay.

Use of stable isotopes to examine the site fidelity of mummichogs (*Fundulus heteroclitus*) in an Atlantic Canadian estuary receiving multiple anthropogenic influences. M.A. Skinner[1], S.C. Courtenay[2], W.R. Parker[3], M.G. Dubé[4] and R.A. Curry[1]. [1]Department of Biology, University of New Brunswick, Fredericton, NB; [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [3]Environment Canada, Environmental Protection Branch, Fredericton, NB; and [4]Environment Canada, National Water Research Institute, Saskatoon, SK.

The goal of this study was to test the hypothesis of low mobility of mummichogs (*Fundulus heteroclitus*) using stable isotope analysis (SIA) of carbon and nitrogen while jointly evaluating the usage of SIA as a method to determine the site-specificity of organisms on a relatively small spatial scale (ca. 10 km). Samples were collected from a section of the upper Miramichi River estuary (MRE), New Brunswick influenced by multiple anthropogenic impacts: two pulp mills and three municipal wastewater facilities. White muscle and bone from mummichogs sampled at 9 sites along the upper MRE (n=198) had overall mean ratios of -21.03±1.45‰ (SD) d^{13}C and 11.37±1.02‰ (SD) d^{15}N. Mean d13C and d^{15}N ratios were significantly different among sites but not between sexes within sites. Mean d^{13}C increased progressively in a downstream direction while two distinct d^{15}N groups representing the northern and southern shores were apparent. These differences appear to be related to the influence of anthropogenic inputs to the system, specifically polycyclic aromatic hydrocarbons and wastewater treatment influences, thus demonstrating the utility of SIA as a method to determine the site-specificity of organisms on a relatively small spatial scale. The scarcity of statistical outliers (2%) during examination of isotopic ratios supports the results of a previous mark-recapture study that showed very few mummichogs (3.1%) in the upper MRE move more than 200 m.

Assessing effects on fish from a stream receiving oil refinery effluent. G. Vallieres, D.L. MacLatchy and K.R. Munkittrick. Department of Biology, University of New Brunswick, St. John, NB.

Estuaries are known to be more complex than freshwater systems, with more mobile fish populations and inconsistent exposure scenarios, increasing the difficulty in linking potential effects on fish health to effluent exposure. In this study, we are assessing fish and fish habitat in an estuarine stream receiving treated oil refinery effluent. The study is comprised of three components which assess (1) fish habitat using water quality parameters, (2) fish communities, and (3) fish health using a small-bodied species effects on growth, reproduction, and condition. Results collected in 2003 show that very low oxygen levels occur during the summer period downstream of the effluent. Moreover, the fish community in the area has been disturbed, diversity and abundance are lower compared to sites for the months of June-November. In addition, mummichog (***Fundulus heteroclitus***) were collected during the summer of 2003 and show similar condition factors among sites, but a much higher liversomatic index (LSI) for individuals collected downstream of the oil refinery effluent discharge site. However, fish residency within the site could not be confirmed using mixed function oxydase (MFO) induction. The work done in 2003 did not permit to link the effects found with the effluent discharge. Consequently, a caging experiment using mummichog was done in the spring 2004 to assess endpoints at the individual level. In addition, fish community and water quality surveys were also undertaken for a 6 month period.

Biology of multi-spawning, small-bodied fish species with sampling considerations for environmental monitoring programs. B.J. Galloway and K.R. Munkittrick. Department of Biology, University of New Brunswick, St. John, NB.

The use of small-bodied fish for environmental monitoring programs has increased over the past few years because they are relatively easy to capture, many species exhibit site fidelity, and they are usually relatively abundant. The main disadvantage of using small-bodied fish for monitoring programs is the lack of basic life-history information, which can hinder study design and data interpretation. The objectives of this study were to collect basic biological information for multiple spawning, small-bodied fish species and provide sampling guidance for the use of these fish in monitoring programs. We examined seasonal changes in condition factor, liver somatic index (LSI), and gonadosomatic (GSI) in four common cyprinids found in Atlantic Canada: blacknose dace (*Rhinichthys atratulus*), northern redbelly dace (*Phoxinus eos*), golden shiner (*Notemigonus crysoleucas*) and the mummichog (*Fundulus heteroclitus*). The correlation coefficient for gonad size versus body size peaked about 6 weeks prior to the first spawning, and declined over the rest of the summer. The variability was highest in some species, like blacknose dace, just before spawning (r2 = 0.43). Correlation coefficients were commonly low (< 0.40) during the summer spawning period, requiring large sample sizes to detect differences between sites. For some species, we were able to minimize this variability by standardizing fish age or body size for comparisons, but this did not work for all species. Ovarian development was more variable in northern redbelly dace relative to all other fish species, and fish with very low GSIs were present during all seasons at a wide range of body sizes. Minimizing sources of natural variability in metrics required for monitoring studies is particularly important since it will increase the probability of detecting impacts associated with contaminant exposure. Some species may not be suitable as sentinel species, given the high variability seen in reference populations during the reproductive season.

Using small-bodied fish in effects-based assessments: interpreting non-lethal data for use in environmental monitoring studies. S. Brasfield[1], M.A. Gray[2] and K.R. Munkittrick[1]. [1]Department of Biology, Univerity of New Brunswick, Saint John, NB; and [2]Department of Biology, Univerity of New Brunswick, Fredericton, NB.

Stressors associated with non-point sources, such as agriculture, are often complex mixtures of chemicals and non-chemical stressors characterized by concentrations of chemicals which are difficult to characterize, and rates and timing of discharges that are difficult to predict. An effects-based approach was used to examine population-level endpoints including survival and reproduction of slimy sculpin (*Cottus cognatus*) in the potato farming belt of New Brunswick. Along the Little River watershed, located north of Grand Falls, differing agricultural intensities at sites along the Little River reach provide a gradient to assess cumulative effects of agriculture. This project is emphasizing non-lethal sampling approaches to using small-bodied fish in small rivers, and a variety of impacts have been documented. Impacts on growth, size distributions, nest densities and fecundity have been documented. Over the past 5 years, year class failures have been documented, and year class strength is negatively correlated with rainfall in agricultural areas. However young-of-year survival was not affected in a year of little rainfall or when summer heavy summer storms preceded pesticide use. Although it is probable that sediment runoff is playing a role in the decreased performance of fish in the agricuultural areas, there are alsoother factors involved with these events, including increases in temperature, nutrients, and pesticides, and changes in the timing and intensity of runoff associated with storm events. Efforts will focus on expansion of the existing knowledge base and development of methods to define cause-effect relationships and an examination of potential solutions to the issues identified.

Complex study designs utilizing small, short-lived species reveal complex patterns: are we gaining insight, or just muddying the waters? C.B. Portt[1], B.W. Kilgour[2], G. Rogozinski[3] and C. Turpin[4]. [1]C. Portt and Associates, Guelph, ON; [2]Jacques Whitford Environment Ltd., Ottawa, ON; [3]Abitibi-Consolidated, Fort Frances, ON; and [4]Abitibi-Consolidated, Montreal, QC.

The Cycle 3 Pulp and Paper Environmental Effects Monitoring (EEM) fish study at the Abitibi-Consolidated Company of Canada mill in Fort Frances, ON, utilized two small, short-lived sentinel fish species, johnny darter (*Etheostoma nigrum*) and mottled sculpin (*Cottus bairdi*). The study design incorporated two upstream reference sites and three sites exposed to mill effluent. The approach was a combined exposure-reference and gradient design. The results indicated a gradient of effects on EEM endpoints through the study area, but the gradient was probably unrelated to mill effluent. The conclusions would have been different if either a typical exposure-reference or gradient design (without reference sites) had been utilized with a reduced number of sites. Obviously, this has important implications with respect to study design and data interpretation, including the determination of whether or not there is a true effect, in the EEM sense. Since similar gradients can be anticipated at many sites receiving effluents that are subject to EEM, this raises important questions from a regulatory standpoint. Possible methods of addressing these questions include adopting different statistical techniques and changing the philosophic framework for defining an effect.

Using environmental gradients to predict reference conditions: a case study with Toronto-area stream fish communities. B.W. Kilgour[1] and L. Stanfield[2]. [1]Jacques Whitford Environment Ltd., Ottawa, ON; and [2]Ontario Ministry of Natural Resources, Picton, ON.

Assessments of stream fish or benthic communities normally involve a contrast of conditions at "test" sites to conditions represented by "regional"-reference sites that are either pristine or "least"-impaired. Identification of reference sites is difficult and normally involves a variety of subjective criteria. The development of reference models for stream fish and benthos in southern Ontario is particularly challenging because there are few undeveloped areas and there is no consensus on criteria for defining the least impaired condition. Rather than identify sites as representing a least-impaired condition, we have developed a series of models that relate the existing biophysical condition of streams (i.e., the fish, benthos and instream habitat) to landscape (i.e., slope, geology, catchment area) and landcover (% imperviousness). Relationships between indices of biophysical condition and imperviousness can be used to "hindcast" or estimate the expected biophysical condition at a variety of imperviousness scenarios. The models cannot be used to predict conditions outside the calibration data range, but this approach does allow us to make use of an impairment gradient, and make predictions with a minimal number of least-impaired sites. The difference between the hindcast reference and present day conditions is an estimate of the present-day impacts.

Examining population-level responses in small-bodied and short-lived fishes: what *Cottus* spp. has taught us. S. Brasfield[1], K.R. Munkittrick[1] and C.B. Portt[2]. [1]Department of Biology, University of New Brunswick, St. John, NB; and [2]C. Portt & Associates, Guelph, ON.

When small-bodied, short-lived fish are used in environmental effects monitoring (EEM) -type studies, samples contain few (typically 1–3) mature age classes. This can require changes in the way that certain endpoints are examined, relative to those from larger, longer-lived species. Our data suggest that the relationship between growth, maturity and female gonad size should be critically examined. In two independent studies using different sculpin (*Cottus* spp.) species a size gap between age classes occurred. The size at which this gap occurred varied between locations, probably as a result of differences in growth rates that occurs. Ovary weights of age 0+ pre-spawners were lower, relative to body size, than among older pre-spawning females. Differences in slopes occurred between samples when the log of ovary weight was regressed against either the log of adjusted body weight or the log of length. It appears that these differences in slopes were probably a result of differences in the age and/or size distributions of mature females, which in turn may have been the result of differences in growth rates and, therefore, should not be interpreted as possible indicators of differences in gonad size among fish of the same size and age. The ability to interpret responses will vary between species, and it is useful to compare the responses of different species in the same area. We feel this information advances interpretation of sculpin responses, and increases awareness for studies involving other small-bodied, short-lived fish that could exhibit similar responses.

Contaminants in Aquatic Systems / Les contaminants présents dans les systèmes aquatiques

Session co-chairs: P.L. Orr and D.M. Whittle

Selenium from coal mining in the Elk River Valley, British Columbia, Canada. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Coal mining in the Elk River Valley, BC, enhances natural release of selenium, resulting in elevated concentrations of Se downstream of the mines. Studies to determine the extent and significance of Se in Valley waters began in 1996. Se concentrations downstream of the five coal mines have increased in some areas. However, the same magnitude of increases has not occurred in lotic (flowing water) fauna, and Se concentrations in fish from lotic areas have not increased from 1996-2003. Although Se concentrations in cutthroat trout and some of their eggs were above concentrations shown to be toxic in other areas with other fish species, a laboratory effects study found that fry hatched and developed normally. Both cutthroat and bull trout populations have increased since 1986. A study of two common waterfowl (American dippers and spotted sandpipers) living in lotic areas found no discernable adverse effects, and Se concentrations in eggs were thresholds at which adverse effects have been documented in other areas. Although lotic areas are most common in the Elk River Valley, lentic (still water) areas may represent the worst case because there is more likelihood of inorganic Se being converted into the much more toxic organic S. A reconnaissance study in 2002 provided no clear evidence of adverse effects from Se in lentic areas. For instance, the most contaminated lentic area was also the most productive area. However, more detailed studies are required before any definitive conclusions are reached. Ongoing and planned studies include determinations of aquatic food webs in both lentic and lotic areas, further monitoring in both areas, and fish and waterfowl effects studies in lentic areas. A human health risk assessment found that there was negligible risk to humans eating fish from the Valley, and benefits from consuming moderate quantities of fish.

Investigation of selenium uptake pathways in lentic and lotic aquatic habitats using stable isotope analysis. P.L. Orr, K. Guiguer and C.K. Russel. Minnow Environmental Inc., Mississauga, ON.

Selenium is an essential micronutrient that exhibits a very narrow margin between nutritionally optimal and potentially toxic concentrations. Egg-laying vertebrates at the top of aquatic food chains appear to be most at risk in environments with elevated aqueous Se concentrations. Available data indicate that Se is more readily accumulated among fish in lentic than lotic aquatic systems and several papers in the literature suggest this may be due to greater uptake through sediment-detrital food chain pathways in lentic than lotic habitats. However, no studies were previously conducted to test this or other potential explanatory mechanisms. This study tested three hypotheses that might account for higher Se concentrations in fish from lentic compared to lotic habitats in a mountain watershed in British Columbia: (1) enhanced uptake by aquatic primary producers, (2) longer food chain length, or (3) greater food web accumulation through sediment-detrital pathways. Stable isotope and Se concentration data demonstrated that Se uptake by aquatic primary producers and the length of the food chains were comparable in lentic and lotic habitats. The study provided evidence that enhanced biotransformation of Se to organoselenium, and subsequent uptake and cycling via sediment-detrital pathways likely account for higher fish tissue S concentrations in lentic than lotic areas.

Temporal trends in mercury and organic contaminants in coho and chinook salmon from Lake Ontario. L.M. Campbell[1], T. French[1], D. Jackson[2], W. Schneider[3] and A. Hayton[3]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Department of Zoology, University of Toronto, Toronto, ON; and [3]Ontario Ministry of Environment, Toronto, ON.

Over the past 3 decades, the Ontario Ministry of Environment has monitored environmental contaminants in sport fish from over 1700 sites across Ontario. The data has been used to determine fish consumption guidelines for people consuming Ontario fish and published in the "Guide to Eating Ontario Sport Fish". We analysed a subset of this large database to determine contaminant trends in coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) from Credit River, Lake Ontario. Credit River flows through Mississauga and is an important sport fishing destination. Overall trends indicate that mercury, total PCBs, mirex and DDT congeners have declined consistently over time. No significant relationships were found with size, condition factor (k) or sex ratios, so we suggest that the decline in mercury and the "legacy" organic contaminants in fish are linked to stronger environmental legislation and increased awareness of the potential risk of those contaminants.

Control of mercury emissions from chlor-alkali plants in India. S. Pandey. Ecotoxicology Laboratory, Hamdard University, New Delhi, India.

Mercury is used in substantial quantities by chlor-alkali plants based on Hg cell technology.

Hg emissions to air, water and products may ultimately contaminate the aquatic food chain and most likely may get converted to methyl-mercury which is harmful to fetuses and young children. In order to avoid adverse impacts on aquatic life and protect human health, efforts were made to control Hg emissions from 16 chlor-alkali plants in India. The paper highlights the various measures taken for reducing the Hg emissions in the environment through Industry-Government partnership program. The measures taken included setting up time bound targets for limiting Hg emissions from the Hg not to exceed 1 gm/ton of product), reduction of Hg in H2 gas at 0.5 gm/ton of the product, demercurization of caustic soda (Hg limit 0.1 gm/ton of product), use of washed salt, capping of existing Hg bearing sludge sites, brine sludge treatment to limit Hg content in brine mud at less than 0.1 mg/l before disposal to landfill sites, total HG release to environment at less than 2 gm/ton by December, 2005 etc. Hg consumption has been reduced from 146 gm/ton to 65 gm/ton and the target is to bring it down further to 50 gm/ton by December, 2005. Similarly, emission levels of Hg at present are 20 gm/ton and the target is to bring it down to 2 gm/ton by December, 2005. As a policy matter, no new chlor-alkali plants are being allowed to be set up in India to avoid any possibility of Hg exposures from such units. Efforts are also being made to persuade the existing industries to switch over to membrane cell technology from the present Hg cell based process. It is expected that about 70% of the existing Hg cell plants may get converted to membrane cell process by the year 2015.

Creososte contamination in the Grey Owl Marina, Prince Albert National Park: PAH and benthos distributions. M.S. Evans and K. Fazakas. Environment Canada, National Water Research Institute, Saskatoon, SK.

The Grey Owl Marina, in Prince Albert National Park (PANP), Saskatchewan was constructed in the early 1960s, consists of 6 piers, and extends over a total area of ca. 27,900 m2. Pilings are constructed of creosote, with preliminary sampling indicating pocyclic aromatic hydrocarbons (PAH) contamination inside the boat slips. PANP is considering dredging portions of the marina and/or reconstruction to allow for more consistent boat access during variable water periods. This study addressees the need for information on sediment quality in the marina and for a contaminated site assessment. A series of sediment core samples were collected under the docks, inside the boat slips, 2 m, 10 m, and 20 m from the outermost pilings. Cores were sectioned at 0-5, 5-10, and 10-15 cm and subsampled for PAHs, grain size, inorganic and organic carbon, and benthos. All surface samples and a subset of the deeper samples were analyzed for PAHs and physical chemical variables; all benthos samples were analyzed. *Interim Sediment Quality Guidelines* for individual PAHs were exceeded at several sites under the piers, inside the boat slips, and 2 and 10 m from the boat slips. Concentrations of several PAHs reached 1,000 μg/kg at some locations and exceeded 100 μg/kg at many locations within 10 m of the pilings. PAH concentrations remained high down to 15 cm close to the pilings and there was a noticeable creosote-like smell and an oily film in many of these sediment samples. Nevertheless, benthos was abundant with no evidence of impaired distributions relative to their location in the marina. The site was classified as moderately contaminated. Dredging and piling removal options must include considerations of elevated PAH concentrations inside and under the boat slips, particularly areas close to the pilings where high PAH concentrations can extend down to at least 15 cm.

Non-destructive sampling for chemical analysis of fish tissues. K. Connors and C.K. Russel.

Minnow Environmental Inc., Missassuga, ON.

Conventional methods for metal analysis in muscle samples usually require fish to be sacrificed due to the relatively large amount of tissue required for analysis. This is can be problematic or prohibited when investigating sensitive or endangered fish populations. Muscle biopsy sampling represents an alternative, non-destructive method for obtaining fish muscle tissue for analysis. To test the comparability of the biopsy method to conventional techniques, Se concentrations in muscle tissue plugs were compared to Se concentrations in muscle fillets taken from the same fish. No significant difference was found between muscle fillet and tissue plug samples in a paired t-test involving data from 15 fish (2-tailed; $p = 0.114$). A strong positive relationship was observed between muscle fillet and plug sample data ($R2 = 0.992$). Based on the results of this study, tissue biopsy samples (plugs) are recommended for analyses that can be completed using a small amount of tissue (e.g., < 50 mg ww).

Assessment of cumulative impacts in three species of fish in a New Zealand river. D.W. West[1], M.R. van den Heuvel[2], N. Ling[1], B. Hicks[1] and L. Tremblay[3]. [1]Centre for Biodiversity and Ecology Research, Waikato University, Hamilton, New Zealand; [2]New Zealand Forest Research Institute, Rotorua, New Zealand; and [3]Landcare Research, Christchurch, New Zealand.

The Waikato River receives numerous point source discharges including municipal sewage, agriculture, geothermal effluent, power plant cooling water, and industrial effluents and it has 8 hydroelectric impoundments in its upper reaches. Resident populations of the exotic brown bullhead catfish (*Ameiurus nebulosus*), indigenous shortfin eel (*Anguilla australis*), and common bully (*Gobiomorphus cotidianus*) were sampled from paired sites upstream and downstream of point-source discharges and from far field reference locations. Relative abundance, age structure, and number and size of eggs per female, and the physiological indices condition factor, organ to somatic weight ratios, gonadosomatic index were measured. Biochemical and chemical indicators of exposure and effect to contaminants such as hepatic ethoxyresorufin-*O*-deethylase (EROD) activity, metals, plasma steroids and bile chemistry were also assessed. Although physiological and chemical measurements show evidence of contaminant exposure differences in relative abundance, population structure, growth rates and condition factors of fish from sites downstream of some discharges suggest populations of catfish particularly benefit from factors (nutrients and higher temperatures) associated with the discharges. Several distinct patterns o population response were observed including increased food availability, food limitation recruitment failure and exploitation. Changes in fish population parameters were likely more strongly influenced by variables such as flow, water level fluctuation, temperature, and exploitation than by contaminants. Resident fish populations appeared to be most influenced by near field point source discharges and local habitat and physical/chemical conditions and cumulative impacts along the river were not obviously manifest. Some observations, such a recruitment failure at one site, remain unexplained and have the potential to be related t chemical exposure.

Zooplankton community structure and aquatic chemistry in northeastern Alberta: potentia sensitivities to increased acidification. M.S. Evans[1], D. Andrews[2], D. Jeffries[3] and P McEachern[4]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Western Resource Solutions, Calgary, AB; [3]Environment Canada, National Water Research Institute Burlington, ON; and [4]Alberta Environment, Edmonton, AB.

Northeastern Alberta has extensive bitumen deposits which are being mined at an accelerating rate. Nitrous oxides and sulfur dioxide are released into the atmosphere during these processes and concerns have been raised regarding the effects of such emissions on nearby lakes and ponds. Here we report on studies conducted to address these issues, as part of the Regional Aquatic Monitoring Program and related surveys. Most of the lakes in northeastern Alberta are shallow (< 5 m) and circumneutral (mean pH 7.8) with calcium bicarbonate the main contributor to total dissolved solids (mean 71.1 mg/L) and buffering. Acid sensitive lakes are few in number and tend to be small, shallow, and located in upland fens. Phosphorus (mean ca. 68 mgP/kg) and chlorophyll (mean ca. 36.8 μg/L) concentrations are high as are dissolved organic carbon concentrations (mean ca. 17.1 mgC/L); nitrogen concentrations are low (ammonia ca. 25.8 μgN/L; nitrate ca. 7.7 μgN/L), suggesting nitrogen limitation. Zooplankton are abundant and dominated by taxa which produce resting eggs, an important adaptation for lakes which may freeze to the bottom and/or become anoxic in winter; lowest densities (mean 4,810/m2) are in lakes with lowest pH values (range 4.0-4.9) versus a mean density of 127,666/m2 in the two lakes with a pH of 5.0-5.2. Because these lakes are highly productive, zooplankton may not become physiologically stressed until pH falls below 5.0. Overall, study lakes have natural features which make them resilient to acidification but possibly sensitive more to increased nitrogen loading which could enhance their productivity.

Chlorine-based disinfection of primary municipal wastewater - a review of practices, efficacy, monitoring and environmental impact. C.S. Bottaro[1] and K.A. Hawboldt[2]. [1]Department of Chemistry, Memorial University of Newfoundland, St. John's, NL; and [2]Faculty of Engineering, Memorial University of Newfoundland, St., John's, NL.

Municipal wastewater treatment plants are typically implemented in phases due to their high cost, as a result many municipalities initially employ primary treatment with plans to expand to secondary and tertiary treatment. However, a high load of pathogenic microbes in primary treatment effluent may require disinfection before discharge. The type of disinfection treatment to use or whether it is required at all is a function of a number of factors including the primary effluent chemistry and the receiving environment. Chlorine disinfection is often the choice due to low cost. However, the questionable efficacy of Cl in some environments and the possible formation of disinfection by-products (DBP) call for a more detailed evaluation of chlorinating primary effluent. Extensive research on the disinfection of drinking-water has shown that chlorination in the presence of humic matter results in production of mutagenic compounds. The chlorination of wastewater from primary treatment is not as well studied, early investigations indicated that ammonia in the wastewater reacts with the Cl to produce inorganic chloramines, which would then act as a disinfectant. However, more recent studies indicate that the Cl also reacts to produce organic chloramines and other carcinogens. Many organic chloramines have already been established as carcinogens, and the reactivity of chloramine with the complex mixture of substances in municipal wastewater (i.e. lipids, humic matter, pharmaceuticals, mixed industrial waste) is unclear. A review of research on the chlorination of wastewater will be presented with a focus on the difficulty of identifying and predicting harmful DBPs.

Food web biomagnification of brominated diphenyl ethers in two Great Lakes fish communities. D.M. Whittle, D.C. MacEachen , D.B. Sergeant and M.J. Keir. Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON.

Surveys of brominated diphenyl ethers (BDE) in fish communities of Lake Michigan and Lake Ontario were completed from 2001-2003. These systems are traditionally the most contaminated among all the Great Lakes with a wide range of toxic substances detected in aquatic biota. The food web studied included fish species lake trout, smelt, alewife and sculpin plus invertebrates *Diporeia*, *Mysis* and plankton. Analyses were completed on samples of individual whole lake trout and 5 whole fish composites of the forage species. A total of 41 congeners were included in the analytical standard. Tetra BDE (#47) and penta BDEs (#99 & #100) were the most prominent congeners in all trophic levels. These are the most common congeners found in a widely used brominated flame retardant. There was significant food chain accumulation with biomagnification factors (BMFs) for forage fish to the top predator species ranging from 3.7-21.9. There was a > 5 fold difference in the concentration of the sum of BDE congeners accumulated in the 3 forage fish species analysed. Age and feeding pattern were significant variables controlling accumulation.

Species differences in the bioaccumulation relative to biotransformation of polycyclic aromatic hydrocarbons. J. Hellou[1], K. Campbell[1], K. Cheeseman[2], A. Gronlund[2], R.D. Guy[1], J. Leonard[3], L. Ramaley[1] and S. Steller[3]. [1]Department of Chemistry, Dalhousie University, Halifax, NS; [2]Department of Biology, Dalhousie University, Halifax, NS; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous contaminants deriving from various combustion and fossil fuel sources (Neff 1979). These molecules are hydrophobic and lipophilic which leads to their absorption to lipid enriched biotic or abiotic particles. Sixteen parental PAH are listed as priority contaminants by many countries, including Canada (CCME 1999), while in the environment, they can also be present as alkylated derivatives, with a variety of structures. Many links in the food chain are exposed to these chemicals and fates will differ with PAH and species (e.g. Varanassi, 1989; Meador et al. 1996). In our continuing studies of the fates of PAH, the metabolism and accumulation of PAH was examined in the laboratory, in fish, whelks and amphipods, to better interpret results obtained in the field.

Two fish species, one marine, winter flounder (*Pseudopleuronectes americanus*), and one freshwater, speckled trout (*Savelinus fontinalis*), were exposed orally to 3 PAH, to determine if dietary uptake is a potential source for bioaccumulation. These three PAH, phenanthrene (PA), pyrene (PY) and fluoranthene (FLU) predominate in many sediments worldwide and are detectable in the water column (references within Hellou et al. 2002 and 2004), as illustrated for sites sampled in Halifax Harbour (Fig. 1). Therefore, organisms living in the water column or on sediments, where particles deposit, will be exposed to PAH and can transfer these contaminants when preyed upon.

In fish, gall bladder bile, livers, other internal organs and muscle tissue were sampled and analyzed at intervals over a period of several days after feeding. Differences between the two fish species were observed in both the nature of the PAH metabolites produced and in their production rate. In trout, pyrene glucuronide was the only metabolite observed. In flounder, phenanthrene glucuronide, pyrene glucuronide and fluoranthene dihydrodiol were observed in significant quantities. In both species, the predominant location of the metabolites was in the gall bladder bile, however, metabolites were also observed in the internal organs. All three parent compounds were detected in the muscle tissue but differences between the two species were

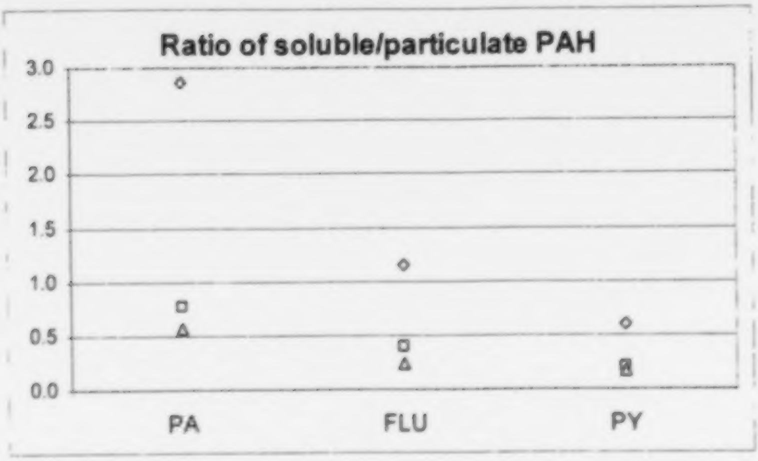

Fig. 1. Example of the partitioning of three predominant PAH in water and particles of three sites in Halifax Harbour. PA, FLU and PY represent phenanthrene, fluoranthene and pyrene, the first is tricyclic, while the other two are tetracyclic.

noted, which implies differences in toxicity. The amount of bile metabolites produced was only a small fraction of the daily dietary dose. Differences in sensitivity reflected in the ease of analysis and detection of PAH or their metabolites are due to analysing a whole gall bladder and only a few grams of other organs, along with the processing of tissues where the biological matrix is more or less complex, leading to more or fewer steps in purifying a sample prior to quantifying the target compounds.

Our results along with earlier studies published in the literature indicate that if the interest is in short-term exposure, then the investigation of gall bladder bile metabolites would be of interest. If exposure was long-term, then this could be deduced by comparing the tissue distribution of PAH. Detectable concentrations in the stomach content of a fish would indicate that ingestion took place, while if PAH are detected in muscle, this would represent a more continuous exposure. Available studies that associate health risk with bioaccumulation or biotransformation, linking chemical and biological endpoints with potential population level effects, are rare (e.g., Heintz *et al.* 1999, Johnson *et al.* 2002) and more are needed. One of the more striking publications regarding an acute effect of non-ionic lipophilic contaminants, i.e. narcosis, makes the link between body burden and the biological effect (McCarthy and Mackay 1993). Chronic exposure can lead to effects under exposure to low concentrations of contaminants and this represents challenges for scientists and managers addressing questions of ecosystem health, cause-effect relationships and taking steps for conservation.

Whelks (*Buccinum undatum*), were collected offshore and fed to mussels spiked with pyrene. This PAH has been used by investigators interested in determining if animals have the ability to biotransform reactive chemicals. The advantage of exposing animals to pyrene is due to the chemical structure of this aromatic compound that leads to the preferential formation of one major oxidised product in many studied organisms. There is a high yield of only one structural isomer of pyrene, 1-pyrenol, in the first phase of the oxidation process (Fig. 2). Two tissues of whelks were analysed over a period of 6 weeks and pyrene and a phase II metabolite detected over time. Therefore, this first study of the fate of a reactive organic chemical in whelks demonstrates that in order to determine if PAH exposure took place, bioaccumulation along with biotransformation should be examined. More studies are needed to determine at what level of exposure, aqueous or dietary, metabolism would be initiated and to link potential effects.

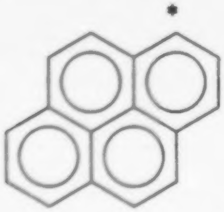

Fig. 2. Structure of pyrene, where the carbon with a star is more readily oxidised.

Amphipods (*Corophium volutator*) were exposed to sediments spiked with PAH. Whole amphipods did not display any biotransformation products over a period of 10 days. The bioaccumulation and depuration of PAH were studied over time to determine the kinetics of uptake and depuration, and how various toxicity tests can be interpreted in time. An example of bioaccumulation results is presented for a series of PAH analysed in amphipods exposed to sediments, where results are expressed in terms of biota sediments accumulation factors (BSAF= concentration in amphipods divided by concentration in sediments, both in dry weight; Fig. 3). BSAF varied between exposures depending on the contamination source, the PAH in question, the time of spring-summer-fall and the size of amphipods. These preliminary investigations of fate were followed by a number of studies associating fate with effects, where lipid content, reproduction and behaviour were examined. Some of these responses were correlated with PAH body burden (In preparation).

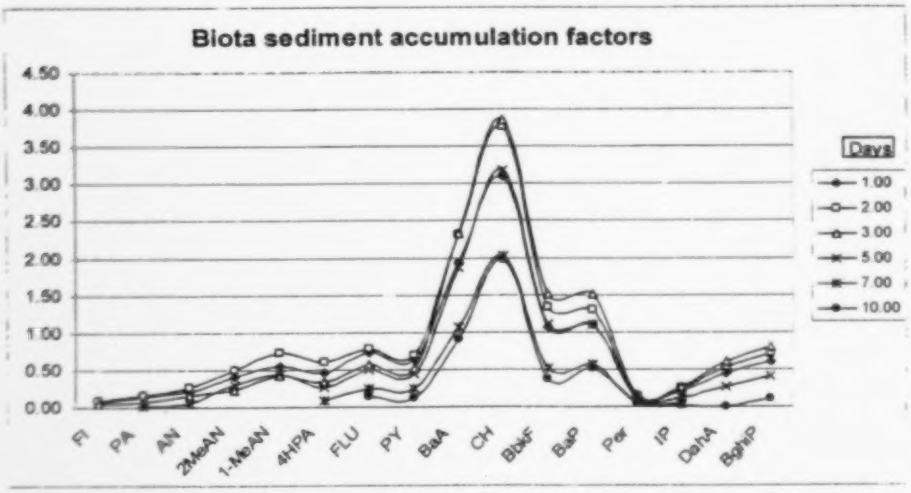

Fig. 3. Example of the kinetics of the bioaccumulation of PAH, with some of the highest BSAF observed in our studies. Fl is fluorene, PA is phananthrene, AN is anthracene, ME is methyl, FLU is fluoranthene, PY is pyrene, with a larger molecular weight PAH having higher or lower BSASF.

The driving force behind our studies is that an understanding of fates leads to a better prevention of risk and interpretation of effects. This goal is better achieved by combining laboratory and field investigations.

Acknowledgements
This research would not have been possible without the financial support of the Department of Fisheries and Oceans, under various National and regional programs.

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Spatial and seasonal dynamics of polychlorinated biphenyl (PCBs) transport and bioaccumulation using freshwater mussels. G.R. Craig[1], I. Middelraad[2], M.H. Salazar[3], S.M. Salazar[3] and C.B. Portt[4]. [1]G.R. Craig and Associates, Schomberg, ON; [2]I. Middelraad & Associates. Guelph, ON; [3]Applied Biomonitoring, Kirkland, WA; and [4]C. Portt & Associates, Guelph, ON.

Caged freshwater mussels, *Lasmigona costata* and *Elliptio complanata*, were transplanted to a shallow, slow flowing watercourse draining an historic transformer manufacturing site containing polychlorinated biphenyls (PCBs). Cages were also placed in the receiving river upstream and downstream of the drainage outfall. Transplant studies were conducted over three years from spring to fall. Consecutive 28 day and longer overlapping deployments of mussels in the system indicated that PCB concentrations in mussels decreased along the route of transport and declined from spring to late summer. Total PCBs accumulation by mussels were influenced byseasonal changes in flow rates in the drainage system and depuration of PCBs during periods of uncontaminated flow. The data suggest that depuration of up to 50% of accumulated PCBs may occur within 9 days of exposure to uncontaminated conditions. Transport of total PCBs was related to the < 14 micron suspended particle size fraction. The data show that even persistent chemicals can take on a seasonal and spatial dynamic depending on local transport conditions and the characteristics of biological receptors.

Nine-year review of Gulfwatch in the Gulf of Maine: Trends in tissue contaminant levels in the blue mussel, ***Mytilus edulis*****, 1993–2001.** L. White[1], P.G. Wells[2], S. Jones[3], C. Krahforst[4], G.C. Harding[5], P. Hennigar[6], G.L. Brun[7] and N. Landry[8]. [1]Ecosystem Research, Halifax, NS; [2]Environment Canada, Canadian Wildlife Service, Dartmouth, NS; [3]University of New Hampshire, Durham, NH; [4]Massachusetts Coastal Zone Management, Boston, MA; [5]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [6]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [7]Environment Canada, Environmental Science Centre, Moncton, NB; and [8]New Hampshire Department of Environmental Services, Concord, NH.

Gulfwatch is an international monitoring program involving three states and two provinces and focused on tissue contaminants in the blue mussel (*Mytilus edulis)*. Data from 1993-2001 has been analyzed to determine spatial and temporal trends and to provide the basis for program re-design. Tissue levels of 9 trace metals, 24 PCB congeners, 24 PAHs and 16 chlorinated pesticides have been monitored from 38 sites. Sampling and processing follow standardized methods and occur annually during the fall. Five benchmark sites are sampled every year, the remainder being sampled once every 3 years rotationally. Four replicate samples of 40 mussels, 20 each for metals and organic chemicals, are collected from each site. Two laboratories conduct the tissue analyses. Where significant temporal trends were detected at benchmark sites, all trends indicated that tissue contamination in blue mussels decreased over nine years (e.g., Pb, Hg, pp'DDE, PCBs). Where spatial patterns were observed, contaminant concentrations tended to be higher in the southern Gulf compared to the northern Gulf, particularly for organic contaminants. Some contaminants showed no spatial pattern, but did indicate areas of highly elevated levels for some contaminants (e.g., Ag, chlordane, and diedrin). Of 16 pesticides, p,p'-DDE was the most frequently detected being found at almost every site. Only four other pesticides are found at > 50% of the sites, while mirex and aldrin were never found. PAH data analyses are underway. Gulfwatch illustrates the role of tissue monitoring in hazard and risk assessment by identifying temporal and spatial trends in ecosystem exposure and exposure variability.

Polychlorinated biphenyls levels in sport fish from Lake Erie. A. Kielaszek[1], T. Franks[2], B. Schepart[2] and P.F. Dehn[1]. [1]Department of Biology, Canisius College, Buffalo, NY; and [2]Waste Stream Technologies, Inc., Buffalo, NY.

Polychlorinated biphenyls (PCB's) are persistent organic pollutants that undergo bioaccumulation throughout the food chain. They represent potential threats to human health via consumption of contaminated food. Several studies have monitored PCB levels in fish from the Great Lakes, but data for Lake Erie fish have been minimal. Chinook salmon (1), lake trout (6), smallmouth bass (10), and yellow perch (8) were collected from Lake Erie in summer 2003.

Skin-off, trimmed fillets were extracted, interferants cleaned up, and analyzed using EPA methods 3540C, 3620B (florisil), and 8082, respectively. PCB's (as Aroclors) were expressed as individual and total Aroclors (μg/kg wet tissue). Aroclor 1260 was found in all of the samples, while A1254 was found in more than half of the samples (salmon, all but 2 of the trout and perch, but only 2 of the bass). Total PCB's were highest in the salmon (431.5±36.1 μg/kg), followed by trout (359.8±130.5 μg/kg), bass (91.6±94.4 μg/kg), and perch (49.5±33.6 μg/kg). All were above the cancer endpoint limits and all but the perch were above the non-cancer endpoint limits based on 4 meals per month, which have been shown to be realistic consumption rates for sports fishers from the Great Lakes. Levels would be significantly higher if skin-on, non-trimmed fillets were analyzed, indicating fish consumption advisories may need to be re-examined for Lake Erie fish, particularly in light of the potential risk to sport and subsistence fishers, the latter of who may consume even greater numbers of fish meals per month. (A. Kielaszek supported by a Merck/AAAS USRP grant to Canisius College).

Mercury in sport fish from Lake Erie. R. Schuster[1], D. Weibel[1], K. Burke[2], L. Shepherd[2], G. Smietana[3], B. Schepart[3], and P.F. Dehn[1]. [1]Department of Biology, Canisius College, Buffalo, NY; [2]Department of Chemistry, Canisius College, Buffalo, NY; and [3]Waste Stream Technology, Inc., Buffalo, NY.

Mercury undergoes bioaccumulation throughout the food chain, and represents a potential threat to human health via consumption of contaminated food. Several studies have monitored mercury levels in fish from the Great Lakes, but data for Lake Erie fish have been minimal. Lake trout (6), small mouth bass (10), and yellow perch (8) were collected from Lake Erie in summer 2003. Skin-off, trimmed fillets were extracted and analyzed using EPA methods 200.7 and 245.2 and 7470A, respectively. Hg levels were expressed μg/kg wet tissue. Mercury was found in all of the samples and were highest in the smallmouth bass (2546±720 μg/kg) followed by perch (1943±676 μg/kg) and trout (690±289 μg/kg) and all but the trout were above EPA's recommended fish consumption limits for methyl mercury (0.23 mg/kg) based on four meals (0.227 kg/meal) per month, which have been shown to be realistic consumption rates for sports fishers from the Great Lakes; indicating fish consumption advisories may need to be re-examined for Lake Erie fish, particularly in light of the potential risk to sport and subsistence fishers,the latter of whom may consume even greater numbers of fish meals per month. (D.Weibel was supported by a Merck/AAAS URSP grant to Canisius College).

Use of small fish in caged field experiments. V.P. Palace. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

A review of the use of caged fish for determining effects from specific effluents and for delineating confounding influences will be presented. Aspects of setting up caged exposure, including selecting fish species, cage construction and cage deployment. Practical advice derived from our metal mining caged exposures will be reviewed. (See Doebel *et al.* in this Proceedings).

Biological Effects / Effet biologique

Session co-chairs: D.L. MacLatchy and W. Robinson

Glutathione *S*-transferases as a pathway for protecting against oxidative stress in fish. E.P.

Gallagher. Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.

Oxidative injury is a major mechanism of injury to mammalian and aquatic species exposed to certain environmental chemicals. Accordingly, the presence and activity of detoxification enzymes that remove reactive oxygen species or breakdown products produced during oxidative injury can be a key determinant of susceptibility to toxicity. Previous studies in aquatic species have demonstrated the presence of pathways such as catalase and superoxide dismutase that detoxify reactive oxygen species. However, little is known regarding the expression or regulation of pathways that detoxify reactive intermediates produced by peroxidation of membrane lipids during oxidative injury. In this regard, largemouth bass (*Micropterus salmoides*), a predatory freshwater fish, have a remarkable ability to conjugate 4-hydroxy-2-nonenal (4HNE), a mutagenic and cytotoxic unsaturated aldehyde produced during the peroxidation of lipids. This ability of bass to detoxify 4HNE can be traced to a glutathione *S*-transferase gene (bass GSTA) whose protein product has a high efficiency towards catalyzing 4HNE conjugation with glutathione. Genomic analysis of GSTA indicates that this gene is part of a GST gene cluster in bass. QPCR analysis of bass exposed to prototypical enzyme inducers suggests that GSTA may function as a housekeeping gene to protect against oxidative stress. A BLAST search of the GenBank database revealed that GSTA is present in several phylogenetically diverse aquatic species, suggesting conservation of an important physiological these studies indicate that GST may represent a major pathway in protecting certain aquatic species against the toxicity of reactive peroxidation products produced during the secondary phases of oxidative injury. Furthermore, it is possible that those aquatic species who express GSTA homologues may have a selective advantage with regards to susceptibility to peroxidative injury. (Supported by NIH P42-ES07375).

Temperature influences polycyclic aromatic hydrocarbon (PAH) solubility, pharmacokinetics, and CYP1A activity in fish. J.A. Dungavell and P.V. Hodson. Department of Biology, Queen's University, Kingston, ON.

Alterations in the rates of uptake and excretion of polycyclic aromatic hydrocarbons (PAHs) by fish can change their toxicities. Rainbow trout (*Oncorhynchus mykiss*) were exposed to retene (7-isopropyl-1-methylphenanthrene) to determine the effects of varying exposure temperature on rates of uptake and excretion. Indices of exposure included liver CYP1A activity measured by ethoxyresorufin-*O*-deethylase (EROD) activity, and concentrations of retene and its metabolites in water, tissue, and bile. When fish were exposed to retene at their acclimation temperature, little difference was observed in the kinetics of PAH metabolism among temperatures ranging from 5-20°C suggesting metabolic compensation for changes in enzyme activity and concentration. However, fish exposed to acute temperature change showed more pronounced differences in uptake and excretion rates. The effects of acclimation temperature on *in vitro* CYP1A of previously induced and non-induced fish were shown by EROD activity and Western blotting. No differences in CYP1A activity or protein concentration were found among the temperatures indicating that compensation occurred at the enzyme level. Acute temperature change is being examined in depth to determine its influence on metabolism of PAH.

Chronic aquatic toxicity of alcohol ethoxylate (AE) surfactants under Canadian exposure conditions. S.E. Belanger[1] and P.B. Dorn[2]. [1]Procter & Gamble, Miami Valley Innovation Center, Cincinnati, OH; and [2]Shell Global Solutions, Inc., Houston, TX.

Abstract

A review of alcohol ethoxylate (AE) fate and effects by the National Guidelines and Standards

Office of Environment Canada will culminate in the issuance of an AE water quality guideline for the protection of aquatic life. AE are nonionic surfactants that are excellent at removing soil and easily formulated into laundry detergents. AE are complex mixtures of various alkyl and ethoxylate chain lengths. Their properties span a range of conditions consistent with the individual contributions of homologues present in the mixtures. Toxicity, biodegradation, and sorption are non-linearly altered by known physical properties of AE. A total of 60 chronic toxicity studies (published and unpublished) of the effects of 25 commercial and pure AE materials on 18 different taxa were collated. EC10, EC20, NOEC and LOEC values on a common statistical platform were generated. AE distributions in Canadian municipal wastewater effluents were monitored to assess relevant environmental distributions. Chronic toxicity structure-activity-relationships (algae, daphnids, and fish) were used to normalize results from tested commercial mixtures to the environmental distributions and then summarized in species sensitivity distribution analyses. Relationships between effect endpoints, species sensitivity distributions and environmental exposures were explored to support site-specific and regional (national) average exposure scenarios. The presence of AE in wastewater treatment plant effluents represents a low risk for Canadian surface waters.

Introduction

Alcohol ethoxylates (AE) are nonionic surfactants that are used in a variety of cleaning applications. AEs may be derived from linearly or primary branched alcohols containing alkyl chains from 9–18 carbon atoms, with C12-C16 being most common (Fig. 1). The parent alcohols may be made from vegetable or animal sources or ethylene oligomerization. Ethylene is oligomerized to form olefins that are then hydroformulated into detergent range alcohols. The alcohols are reacted with ethylene oxide (ethoxylated) to form AEs with a typical range from EO-0 to EO-18. These nonionic surfactants, are low foaming, are nonpolar and dissolve in water without forming ions, which makes them excellent for soil removal. They are easily formulated into laundry detergents. Their properties span a range of conditions consistent with the individual contributions of homologues present in the mixtures. Commercial and environmental mixtures of AE are a combination of all permutations of alkyl and ethoxylate chain lengths.

Sorptivity, toxicity, and biodegradability of AE are affected by each homologue's hydrophobicity (Van Compernolle *et al.* in review; Boeije *et al.* in review, Wind *et al.*, in review). As alkyl chain length increases and ethoxylate chain length declines, the hydrophobicity of the AE increases (Roberts 1991). Because AE are surfactants that desire to rest at the water:octanol interface, direct measurement of log Kow is inaccurate although techniques exist to mathematically estimate log Kow (Leo and Hansch 1979). By these methods, log Kow values for AE homologues range by orders of magnitude from <2 to approximately 8. Commercial distributions generally range from 3 to 6.

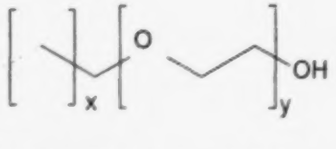

Fig. 1. Generic structure of alcohol ethoxylate (AE) a nonionic surfactant. The hydrophobic moiety or alkyl chain will typically range (x) from 7-16 (9-18 carbons in total) and the hydrophilic moiety or ethoxylate chain will range (y) from 1-18.

Environmental mixtures of AE are different than the commercial distributions which are released down the drain due to the differential effects of sorption and biodegradation of each homologue in the mixture (Wind *et al.* in review, Eadsforth *et al.* in review). This presents a particular challenge to assessing the potential environmental effects of AE. An industry task force, sponsored by the industry association ERASM (Environmental Risk Assessment Management, www.erasm.org) under the auspices of AIS (Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien) and CESIO (Comité Européen des Agents Surface et de leurs Intermédiaires Organiques), was formed following the Dutch review of surfactants in the 1990's to address remaining questions regarding the environmental safety of these high volume chemicals, including AE (van de Plassche *et al.* 1999, AISE 1996). Since that time, other nonionic surfactants commonly used in some applications have come under environmental scrutiny, including nonylphenol ethoxylates (NPE) and its degradation product nonylphenol (NP). A portion of this activity led to the designation of NPE as CEPA Toxic in 2000 by Health Canada and Environment Canada. AE has been used in cleaning applications for decades; however, evaluation of AE in the Canadian environment on an in-depth level has not been done.

A monitoring exercise was completed by industry in 2003 (Eadsforth *et al*, in review) to characterize the concentration and homologue distributions of AE in Canadian wastewater treatment plant effluents discharged to rivers. This paper summarizes aspects of a re-assessment of the AE chronic aquatic toxicity database that includes numerous studies that followed the environmental risk assessment of surfactants in Holland. The objective was to collate and re-analyze data using a consistent statistical approach and to place the effects data into a Canadian context.

Materials and Methods

Collation and Evaluation of Data

Chronic toxicity data were collected from the published literature and from industry archives. Numerous stream mesocosm studies were conducted by the surfactant industry in the 1990's in response to the Dutch surfactant assessment (Dorn *et al.* 1996, 1997a, 1997b, Belanger *et al.* 2000, Wong *et al.* 2004) that included a wide array of new single species toxicity investigations for comparative purposes. Many industry reports were also evaluated provided through the European surfactant industry association, ERASM. Study quality (older and new published data and industry reports) was determined by comparing adherence to published protocols and quality criteria set by the Canadian Council of Ministers of the Environment for establishing national water quality guidelines (CCME 1999).

All raw data were collected whenever possible. A total of 60 studies of 25 different AE compounds were assessed. Raw endpoint and exposure concentration data were available for 48 studies. For the remaining 12, previously published analyses were used. For the 48 studies that were re-analyzed, preference was given to quantifying chronic EC10 and EC20s with 95% confidence limits per endpoint by the method of Bruce and Versteeg (1992). This method is a nonlinear estimation technique that uses a convergence criterion. If the regression did not converge to an EC10 or EC20 then parametric one-way analysis of variance followed by Dunnett's multiple range test at $\alpha = 0.05$ was used.

Normalizing Aquatic Toxicity Data

Alcohol ethoxylates are mixtures of numerous combinations of alkyl and ethoxylate chain lengths. The hydrophobicity of AE homologues is a consequence of both alkyl chain length (increases hydrophobicity) and ethoxylation (lowers hydrophobicity). Hydrophobicity can be indirectly modeled as a function of calculated octanol:water partitioning and has been correlated to toxicity, sorption, and biodegradation (Boeije *et al.* in review, Van Compernolle *et al.* in review). Chronic ecotoxicity has been successfully modeled using simple concentration addition to produce QSARs for algae, daphnids, and fish (Boeije *et al.* in review, Wind and Belanger in preparation). Because the various AEs that have been tested for ecotoxicity have been shown to vary based on their hydrophobicity, the QSARs can be used to normalize results to a common structure to allow inter-species comparisons of sensitivity and develop species sensitivity distributions. This process was developed by van de Plassche et al (1999) for use in past AE environmental risk assessments, although with less sophistication. The normalization per test can be accomplished using the following formula:

$$\text{normalised } EC_{env} = \text{reported}EC_{test} * \left(\frac{\text{predicted } EC_{env}}{\text{predicted } EC_{test}} \right)$$

where,

normalized EC_{env} = normalized effect concentration for environmental distribution,
reported EC_{test} = effect concentration from a toxicity test on a commercial distribution,
predicted EC_{env} = effect concentration from a QSAR based on environmental distribution,
predicted EC_{test} = effect concentration from a QSAR based on a commercial distribution.

Using this procedure, data from tests conducted on various AEs can be compared and summarized. Once data were normalized, a geometric mean of the response of the most sensitive endpoint per species was calculated to represent the overall response of the taxon.

A series of recent studies has shown that AE environmental exposure is a critical factor to consider in the normalization process. In the laboratory, exposure is maximized because of the test conditions. However, toxicity data can also be normalized to environmental distributions as well. Here, the profile of the AE distribution, also known as the environmental fingerprint, is different from that in commercial distributions because all forms of commercial products contribute to an environmental distribution and the relative contribution of individual AE homologues to the distribution is vastly altered by waste treatment processes. Further, additional surfactant and non-surfactant sources can contribute to the profile including natural sources of fatty alcohol (Leeming *et al.*, 1994), consumer product uses of fatty alcohol (Modler *et al.*, 2002), and from biodegradation of closely related alcohol-based anionic surfactants (Modler *et al.* 2002). When dealing with normalization of environmental fingerprints it is appropriate to partition these other sources. Wind *et al.* (in review) demonstrated that the majority of alcohol measured by the AE analytical method is not derived from AE and therefore developed a procedure to partition the appropriate amount of fatty alcohol to the AE environmental fingerprint, also called the alcohol cap. Further, the hydrophobic nature of AE makes these compounds sorptive to organic carbon and suspended solids thereby reducing bioavailability. Van Compernolle et al. (in review) developed an AE-specific sorption QSAR to adjust the bioavailable fraction from environmental fingerprints. The alcohol cap and bioavailability adjustments were independently considered when normalizing toxicity data to a series of environmental fingerprints from 8 Canadian wastewater treatment plants discharged to rivers (Eadsforth *et al.*, in review).

Statistical Analyses

The revised effect database was evaluated in various ways to evaluate the effect of revising the effect conclusions on the risk assessment outcomes for AE. Chronic EC10 and NOECs as well as EC20s and LOECs were directly compared by regressing the point estimates of effect (NOEC, LOEC) versus the continuous estimate of effect (EC10, EC20). SAS (1999) was used in all statistical analytical applications. Species sensitivity was also evaluated in various ways. AE acts by a non-polar, narcotic mode of action and the effect of AE across trophic levels should be similar, therefore, we evaluated the categorization of species by trophic level against their predicted, normalized effect conclusions. Lastly, because the database is large, predictions of a low risk environmental concentration should not be done by simple extrapolation factors (e.g., a factor of 10 applied to the lowest chronic value). Instead, species sensitivity distribution analysis was performed by the methods of Aldenberg and Slob (1993) using the extrapolation software of van Vlaardingen *et al.* (2003) to predict the HC5, the hazardous concentration protective of 95% of species in the aquatic ecosystem.

Results and Discussion

Effect and Endpoint Conclusions

Chronic EC10s and EC20s could be calculated in the majority of cases at 78.3 and 80.0 % of the time, respectively. NOECs and LOECs were calculated in 83% of the cases. Instances where either NOEC or LOEC conclusions were reflected as either less than or greater than values were not included in these comparisons (in other words, at least one concentration needed to exhibit an effect to define the LOEC and one concentration needed to be not significantly different from the control to define the NOEC). In 55% of the cases all four effect conclusions (NOEC, LOEC, EC10 and EC20) were statistically valid. In the majority of cases where comparisons could not be made, the primary reason was the inability to access original data to calculate ECx values (14 of 60 cases, or 23% of the time). Because of the breadth and statistical validity of the database, cross-comparisons of EC10 – NOEC and EC20 – LOEC relationships were viable. The chronic EC10s for AE have a robust relationship to NOEC values across several orders of magnitude. The slope was approximately 1 (0.891) with an intercept near 0 (0.053) and a significant amount of variation was explained ($p < 0.001$) (Fig. 2). Similarly, the EC20 and LOECs had a robust relationship. The implications of both relationships are that historical assessments based on analysis of variance (conclusions of NOECs and LOECs) will not change greatly if the assessment considered point estimates (ECx values). This is not to say that all individual species conclusions would remain the same, but the overall trends would be similar. Given the advantage of ECx evaluations over analysis of variance it seems reasonable to base future environmental assessments on the ECx.

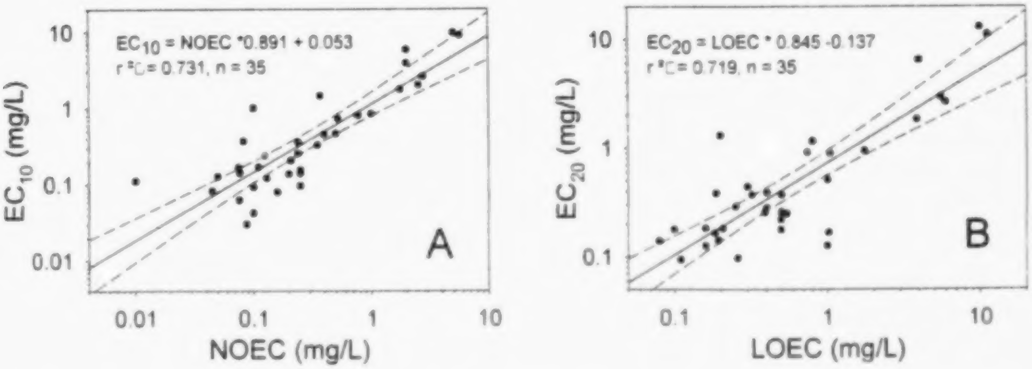

Fig. 2. Relationships of point estimates of effect to threshold conclusions from analyses of variance for alcohol ethoxylate chronic toxicity data.

Chronic toxicity data were normalized to a series of environmental fingerprints (complex commercial distributions and those representative of AE in Canadian surface waters) (Fig. 3). The distribution of sensitivity across trophic levels by taxonomic grouping (algae, invertebrate, fish) did not identify uniquely sensitive or tolerant groups (ANOVA F-statistics ranged from 1.62-1.93, p-values ranged from 0.14-0.21). Species sensitivity distributions corroborated the random nature of sensitivity per trophic level (Fig. 4). Fig. 4 presents species sensitivity distributions (based on chronic EC10s) for the average, full environmental fingerprint of AE from Canadian wastewater treatment plant effluents before (A) and after (B) adjustment to account for the alcohol cap and sorption of homologues to wastewater solids. It is interesting to note that the two most and two least sensitive taxa were consistent in their placements in the distributions.

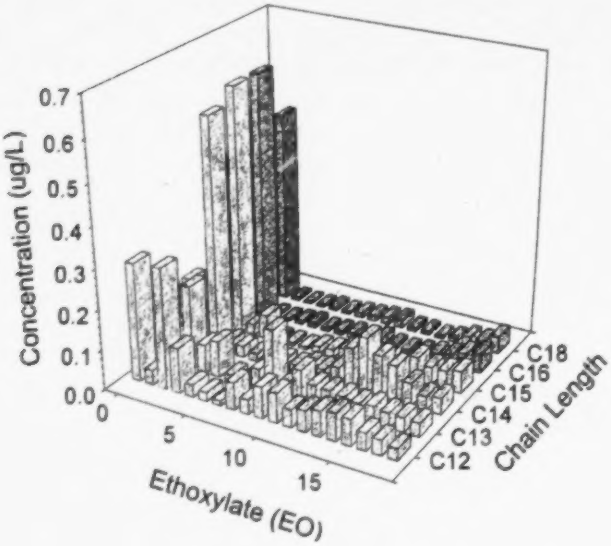

Fig. 3. Example environmental distribution of alcohol ethoxylate. The distribution presented here is the full distribution for 8 monitored Canadian wastewater treatment plants without the alcohol cap imposed to correct for alcohol that should not be associated with the AE distribution.

The HC5 determinations for the above distributions were 10.7 (95% confidence limits 4.8-18.3) and 37.9 (95% confidence limits 17.9-62.4) μg/L, respectively. It is worthwhile noting that *Chlorella vulgaris* was the least tolerant taxon and played a measurable role in the HC5 determinations. If Chlorella is deleted from the data set, the HC5 increases from 37.9 to 49.7 μg/L; however, the 95% confidence limits decline substantially to 28.2-72.4 μg/L. The ratio of lower to upper confidence limits as a measure of overall uncertainty declined from 3.48 to 2.57 when the HC5 is calculated including and excluding *Chlorella*, respectively. While it may seem counterintuitive that deletion of the most tolerant taxon in the database increases the HC5 and narrows uncertainty, the relationship can be directly tied to the presence of the variance in the effect concentration being considered in the denominator of the HC5 statistic. With the deletion of *Chlorella*, overall variance of the EC10 values in the data set declined, thereby reducing uncertainty (Posthuma *et al.*, 2002). By retaining the tolerant taxon in the risk assessment, a lower HC5 is the result, hence the statistic is more conservative.

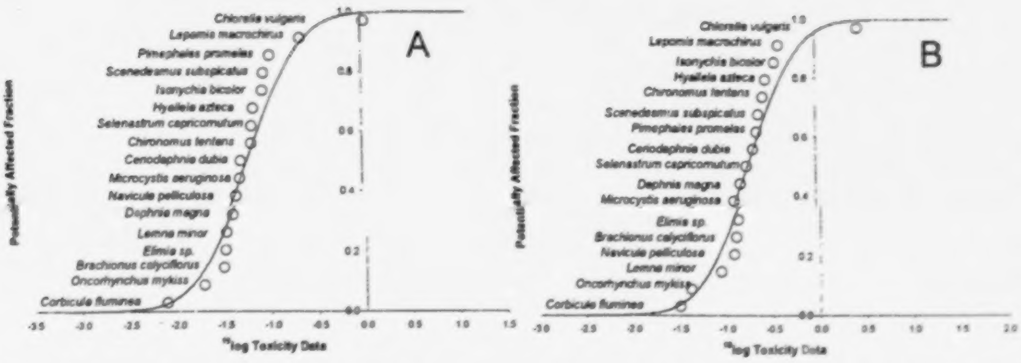

Fig. 4. Species sensitivity distributions normalized to the Canadian average distribution fro wastewater treatment plants. Normalizations were based on the chronic EC10s per test and specie Fig. 4A represents the order of sensitivity when the full distribution is considered and Fig. 4B is th order when the distribution is corrected for the alcohol cap and bioavailability.

Table 1. Mean responses of the most frequently evaluated taxa following normalization to a s environmental fingerprint (average Canadian wastewater treatment plant effluent) without considerin the additional exposure modifiers (alcohol cap and sorption).

Taxon	Common Name	N	EC_{10} Mean (µg/L)	EC_{10} SD (µg/L)	EC_{10} CV (%)
Scenedesmus subspicatus	Green algae	10	69.0	186.3	270.2
Brachionus calyciflorus	Rotifer	6	35.3	21.1	59.7
Ceriodaphnia dubia	Daphnid	4	57.4	28.4	49.5
Daphnia magna	Daphnid	9	48.9	43.0	87.9
Pimephales promelas	Cyprinid fish	14	146.1	130.0	89.0

Several species have been repeatedly tested with various AEs representing differe hydrophobicities. Following normalization chronic results from these different tests can be compar on an equal footing. Arithmetic mean responses for the most commonly tested species (n >3) a summarized in Table 1 below. Data for *Scenedesmus* is the most variable, likely reflecting the fa that maintaining exposure concentrations for these substances can be difficult. Invertebrate and fi data have approximately the same levels of inter-test variability as expressed by the coefficient variation.

The effect of potential exposure modifiers to the Canadian effluent fingerprint on speci sensitivity distribution predictions suggests that both the alcohol cap and bioavailability are importa to consider (Table 2). Predicted HC5s based on the chronic EC10s ranged from 7.9-16.4 µg/L wi no alcohol cap or sorption corrections imposed with the HC5 of the Canadian average distributio being 10.7 µg/L. Because the average environmental distribution is determined on a per homolog basis across all 114 components the HC5 of the average distribution is different than the arithme average of the individual site HC5 values. HC5 values increase to 24.7 and 17.9 µg/L by includi the alcohol cap or sorption corrections, respectively. By modifying environmental exposure using bo the alcohol cap and sorption, the HC5 increases to 37.9 µg/L. If the effect endpoint is increased the chronic EC20 a similar suite of observations can be made except the HC5 values are measurab larger. In both circumstances, using the exposure modifications increases the HC5 by a factor of 3-5 times relative to the fully monitored environmental distribution.

Table 2. Effect of the alcohol cap and sorption (bioavailability correction) on normalized aquatic toxicity data and the resulting species sensitivity distributions. Values below are the computed HC5 (μg/L) or concentration that is predicted to protect 95% of species in the environment.

Site	No Cap No Sorption	With Cap No Sorption	No Cap With Sorption	With Cap With Sorption
	HC_5 Based on EC_{10}			
Vernon, BC	14.8	35.8	27.8	48.8
Kelowna, BC	16.4	36.4	24.6	44.0
Toronto, ON	14.9	41.9	22.7	46.6
La Prairie, QC	12.2	31.4	22.9	42.7
Victoriaville, QC	12.0	35.2	24.6	42.9
Paris, ON	13.7	21.8	21.7	32.6
Cardston, AB	7.9	33.7	12.8	39.6
Waterloo, ON	11.1	22.2	18.0	29.8
Canadian average	10.7	24.7	17.9	37.9
	HC_5 Based on EC_{20}			
Vernon, BC	20.3	51.4	39.6	72.1
Kelowna, BC	22.6	52.2	35.3	65.3
Toronto, ON	20.6	60.6	32.1	68.9
La Prairie, QC	16.7	44.8	32.5	63.4
Victoriaville, QC	16.3	50.4	34.5	63.1
Paris, ON	18.9	30.4	30.7	47.2
Cardston, AB	10.8	49.2	18.2	59.6
Waterloo, ON	15.3	31.7	25.9	44.3
Canadian Average	15.3	52.3	26.1	83.8

Belanger *et al.* (in review) developed a full aquatic environmental risk assessment of AE based on US. European, and Canadian monitoring. A new approach to assess the risk of AE in the aquatic compartment was proposed based on the mixture toxicity concept using concentration addition (Boeije *et al.* in review). Risk characterization was accomplished by combining effluent monitored values of AE without in-stream dilution with predicted HC5s of pure homologues and expressed as Toxic Units. In this assessment, Measured Effluent Concentrations were equated with Predicted Environmental Concentrations and HC5s with Predicted No Effect Concentrations (PNECs) on a homologue-by-homologue basis. TUs ranged from 0.011 to 0.184 for 29 sites around the world indicating an extremely low level of risk in surface waters. In-stream dilution, settling, and biodegradation would reduce exposure and Toxic Units further following entry of wastewater effluents into receiving waters.

Acknowledgements

The authors gratefully acknowledge the efforts of the ERASM Complex Substance Aquatic Risk Assessment Task Force in developing many of the concepts and data reflected in this paper. K. Potter, U. Schneider, and D. Spry of Environment Canada National Guidelines and Standards Office provided much input and posed questions that resulted in the formulation of the TU approach by ERASM to address emerging needs voiced by Environment Canada.

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Occurrence and persistence of Cry1Ab genes in the aquatic environment. F. Gagné, C. André and C. Blaise. Centre Saint-Laurent, Montréal, Qc.

The application of *Bacillus thuringiensis* (Bt) and the practice of growing genetically-modified crops are two of the methods currently being used to control crop-eating insects. The increasing use of these biotechnology products may lead to an increase in Cry1Ab genes both in terrestrial and aquatic environments. The aim of this study was therefore to quantify the levels of Cry1Ab genes in the environment and persistence in freshwater surface waters and sediments. Agricultural soils, sediments and surface waters were spiked with either Dipel® biopesticide or Bt-corn genomic DNA and allowed to incubate at 15°C for several days. Cry1Ab gene concentrations were determined by real-time PCR. In addition, surface water, soils and sediments were sampled in streams and rivers nearby a field growing genetically-modified corn expressing Cry1Ab. The results show that Cry1Ab genes are found in either drain or surface waters and sediments for more than 3 km downstream the field. The proportion of Cry1Ab from Bt-corn could represent up to 40 % of total Cry1Ab as determined by mixed primers real-time PCR methodology. In spike samples with genomic DNA, the half-life (t1/2) of Cry1Ab gene was persistent for 10-14 days in surface waters and removal of microorganisms by filtration increased the t1/2 for Bt-corn only. However, in sediments spike with genomic DNA, an increase in Cry1Ab was observed with a generation t1/2 of 14.3 days for both Bt and Bt-corn. It appears that long-term contact of DNA to sediments leads to amplification of Cry1Ab gene but this effect was not related with genetically-modified organisms. These results suggest that Cry1Ab genes from Bt-corn and Bt are found in aquatic ecosystems nearby Bt-corn fields. These genes have the ability to amplify in spiked sediment samples in the laboratory regardless of their origins. Further research are planned to quantify their gene expression (mRNA) in aquatic environments.

Expanding the application of field-based mesocosms for life cycle studies with benthic invertebrates and partial life cycle studies with fish. M.G. Dubé[1], C. Rickwood[2], K.A. Hruska[2], D.L. MacLatchy[3] and N. Glozier[1]. 1Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [3]Department of Biology, University of New Brunswick, St. John, NB.

Artificial stream systems have been developed in Canada for over a decade to assess the effects of complex effluents (e.g., pulp and paper, metal mining, municipal sewage) on benthic invertebrate communities and fish populations. Since 1990, field-based studies have been conducted on at least 20 occasions in 4 Canadian provinces in freshwater, estuarine and marine environments. Studies have been conducted on single benthic invertebrate populations (e.g., mayflies, chironomids), benthic invertebrate communities, and using 7 different small-bodied fish species including juvenile Atlantic salmon, mummichog, fathead minnow, longnose dace, pearl dace, and creek chub. These research efforts have resulted in the acceptance of artificial stream systems as alternative approaches to use in different phases of the legislated Canadian Environmental the artificial stream systems focused exclusively on the effects of effluents on endpoints such as survival, gonad size, liver size, reproductive hormone levels, and growth after a 30-60 day exposure period. Benthic invertebrate studies examined changes in community structure in exposure streams relative to after a typical 30 d exposure. In an effort to develop a better understanding of reproductive effects, a significant effort has been made since 2002 developing the systems to assess lifecycle and partial lifecycle endpoints in both a benthic invertebrate (*Chironomus tentans*) and the fathead minnow (*Pimephales promelas*) after exposure to pulp mill and metal mining effluents in both field and laboratory studies. These recent advancements will assist with understanding the linkages between individual level changes with stress to those expressed at the population level as well as in Environmental Effects Monitoring investigation of cause studies.

Is the Burrard inlet homogenous? What the mussels are telling us. S.D. St-Jean[1], F. Bishay[2] and P. van Poppelen[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Greater Vancouver Regional District, Burnaby, BC.

The Greater Vancouver Regional District wastewater treatment facilities at Lion's Gate are serving a population of around 160,000, cover an area of more than 8,760 ha via a separate sewer system and have an average dry weather flow of 80 MLD (or 92 MLD average flow in 2003). The treatment process is primary; sludge is digested on site. The effluent discharges into Burrard Inlet at First Narrows, and the plume disperses either east or west into the inner harbour or outer Burrard Inlet depending on the tide. Burrard Inlet is also influenced by the Fraser River and other freshwater discharges, spoil and dredged material dumping, fishing, shipping and industrial activities. Evidence of the effluent plume in the sediments has not been identified, possibly due to the dynamic nature of the immediate receiving environment resulting in thorough mixing such that the effluent plume cannot be distinguished. To verify this hypothesis, we caged blue mussels (*Mytilus edulis*) and pacific mussels (*M. trossulus*) at one to three depths stations at six sites in Burrard Inlet and four Reference sites from February to December in 2003, and monitored four wild population sites for a total of 19 stations. We measured condition indices, growth, survival, reproductive effort, leukemia and certain aspect of the immune system in both wild and caged mussels. The results show that the inlet is not homogenous and that zones of elevated stress could be distinguished. These results and their implications will be discussed.

Importance of dietary uptake on metal toxicity to *Hyalella azteca*. A.L. Wallace[1], U. Borgmann[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

In recent years there has been an increase in interest on the importance of dietary uptake of metals in aquatic organisms. Numerous experiments have been conducted on various organisms, however few have examined the effects of dietary uptake on metal toxicity to sensitive species such as *Hyalella azteca*. A series of bioaccumulation and toxicity experiments exposing *H. azteca* to cadmium contaminated *Chlorella fusca* will be conducted to determine the relative importance of dietary and water sources of Cd to *Hyalella*. Various endpoints such as survival, growth and potentially reproduction will be examined. Critical body concentrations obtained from dietary exposures will be compared to those obtained from water-only exposures. Data from this study will assist in creating more complete and meaningful pharmacokinetic models for *Hyalella*. *Chorella* has been grown in a medium without EDTA over a range of Cd concentrations for a period of one week. Methods have been established to harvest the cells and dry them in an oven, creating flakes which can be fed to *Hyalella*. Preliminary experiments suggest that *Chlorella* is a suitable food source for *Hyalella*. It has also been established that relatively small amounts of Cd leach out of the *Chlorella* into the exposure water during feeding. EDTA must be added to the feeding exposures in order to ensure Cd uptake is solely through dietary sources. Feeding exposures using *Chlorella* grown at various Cd concentrations resulted in mortality and reduced growth of *Hyalella*.

Further characterization of a metal-binding, histidine-rich glycoprotein (HRG) from the blood of marine bivalve molluscs. W. Robinson[1], M. Sugumaran[2], G. Wallace[1], A. Abebe[2] and S. Catanzano[2]. [1]Department of Environment, Earth and Ocean Science, University of Massachusetts, Boston, MA; and [2]Department of Biology, University of Massachusetts, Boston, MA.

While limited work has been done on metal-binding transport proteins in the blood of marine bivalves, these proteins could prove to be useful and facile biomarkers of metal exposure and/or toxicity. One such protein, Histidine-rich Glycoprotein (HRG), was isolated and characterized from the blood of the mussel *Mytilus edulis* (Nair and Robinson, 1999, 2001a,b). Immobilized Metal Affinity Chromatography (IMAC; iminodiacetateSepharose 6B) indicates that HRG binds the Class A metals Ca and Mg, the Class B metals Hg and Pd, and the Borderline metals Cd, Ni and Zn. Incubations with N-glycanase and O-glycanase demonstrate that HRG is N-glycosylated and that the polymorphic nature of the protein (MW 29, 35, 37 and 39 kDa) is due to varying amounts of carbohydrate. Glycosylation is needed for metal binding; de-N-glycosylated HRG will not bind to IMAC columns. ELISA assays, using polyclonal antibodies against purified HRG, show that HRG is the predominant protein in mussel blood (~60 % of plasma proteins, by weight). Temporally, HRG concentrations tend to be higher during the warmest months of the year (< 2-fold range). HRG concentrations vary only slightly (1.4-fold range) among 7 Massachusetts coastal locations, and may not be related to metal pollution. Thus, HRG may not prove to be a good exposure biomarker. Evidence indicates that the mussel HRG that we are working on is the same mussel protein described by Renwrantz *et al.* (1998) as SPB1, Hattan *et al.* (2001) as EP glycoprotein and Schneeweiss *et al.* (2002) as HIP. (Funded by National Sea Grant 5710001173).

Influence of hardness on aqueous uranium toxicity. K.C. Serben[1], C.V. Eickhoff[1], G.A. Bird[2] and S. Munger[2]. [1]Vizon SciTec, Vancouver, BC; and [2]Canadian Nuclear Safety Commission, Ottawa, ON.

Uranium (U) toxicity was measured at low alkalinity (5-14 mg/L as CaCO3) and various hardnesses (5-252 mg/L as CaCO3) for six freshwater species using Environment Canada test methods. Tests included 7-day survival and growth tests with fathead minnow (FHM) (*Pimephales* *promelas*), 96-h acute lethality and 30- and 31-day embryo/alevin (E/A) tests with rainbow trout (RT) (*Oncorhynchus mykiss*), 3-brood survival and reproduction tests with *Ceriodaphnia dubia*, 14-day water-only survival and growth tests with *Hyalella azteca*, 72-h growth inhibition tests with *Selenastrum capricornutum*, and 7-day static growth inhibition tests with *Lemna minor*. *H. azteca, C. dubia, and S. capricornutum* were the most sensitive species to U with LC/EC50 (survival, reproduction, and growth) that ranged between 0.017-0.34 mg U/L. moderate sensitivity to U with EC50s for viability between 0.46-0.64 mg U/L. In the FHM tests, 7-day LC50s were 1.5-2.1 mg U/L. RT fry and *L. minor* were relatively tolerant to U with LC/EC50s greater than 2 mg/L. A decrease in toxicity with an increase in hardness was only observed in the *H. azteca, L. minor* and RT E/A tests. This was most pronounced for *H. azteca* where the 14-day LC50 increased from 0.017 mg U/L in soft water to 0.34 mg U/L in hard water, a decrease in toxicity by a factor of 20. Toxicity remained relatively constant with increased hardness for the other species/tests.

Are fish in areas of concern (AOCs) on the Canadian side of the Great Lakes exposed to estrogens? J.P. Sherry, C. Tinson, M.E. McMaster and S.B. Brown. Environment Canada, National Water Research Institute, Burlington, ON.

As part of a broader assessment of wildlife health in areas of concern (AOCs) on the Canadian side of the Great Lakes we have examined feral fish for evidence of exposure to both environmental estrogens (EEs) and anti-estrogens. For the purposes of this study we divided each AOC into upstream, impact zone, and downstream sites. At each site we sought to capture 20 adult male and female specimens of a bottom dwelling and a water-column dwelling fish species. We were able to meet our quota at most, but not all, AOCs. After a general health assessment a blood sample was taken from each fish. The plasma was separated and stored in liquid nitrogen prior to analysis. Vitellogenin (Vg) was measured by means of a sensitive electrophoresis technique which shall be described in the poster. Presence of Vg in the plasma of male fish indicates exposure to EEs. A decreased level of plasma Vg in female fish from the impact zone compared with levels in females from the reference location indicates that the fish have possibly been exposed to anti-estrogens. We present the results for the Wheatley Harbour, Detroit River, and St. Clair River AOCs. The data suggest that feral fish at the Wheatley Harbour and Detroit River AOCs have been exposed to EEs. We also present evidence that fish at the reference site for Wheatley Harbour may have been exposed to EEs. This is a preliminary study. Further research will be required to confirm the observed effects and to determine possible causes.

Microcystin-LR effects on the cytoskeleton of the human hepatoma (HepG2) cell line. J. Cercone, E. Welhhofer and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Cyanobacterial blooms of *Microcystis aeruginosa* have been reported in Lake Erie since 1996 and represent a potential health threat to animals and humans, as they produce a potent hepatotoxin, microcystin-LR (MCLR). MCLR inhibits protein phosphatases which are implicated in cytoskeleton regulation. The cytosketelon is essential for the maintenance of cell shape, motility, organelle movement and cell division. Age of cell and MCLR effects were assessed morphologically. Age effects included increases in actin intensity and a greater shift in the orientation of microtubules from a radial to parallel arrangement in older cells. MCLR effects included significant increases in the presence of microspikes and shifts in microtubular orientation in older cells, the latter of which were greater than those observed due to age. These effects suggest changes in the levels or re-organization of F-actin, and a major reorganization of tubulin in its polymerized form, all of which can impact cell shape, cell-cell interactions, and cellular organization. These morphological changes have been associated with altered cell function, which can lead to toxicity. (J. Cercone supported by HHMI-

USEG to Canisius College).

Wood leachate concentration in waters from the Pacific coast, are there effects on the health of mussels, *Mytilus trossulus*. S.D. St-Jean[1], M. Kohli[1] and P. van Poppelen[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Greater Vancouver Regional District, Burnaby, BC.

In 2004, water samples from Howe Sound on the west coast were taken one meter below the water surface and as well, one meter above the sediment bottom adjacent to a log boom storage area. These water samples were analyzed for several typical wood leachates, such as pinosylvins. In addition, mussels were caged near these operations and their health was evaluated using both condition indices, such as gonadosomatic index, growth and condition factors, as well as for the presence of leukemia, previously reported to be associated with pulpmill effluents. The analytical results have shown the presence of several compounds associated with log boom storage. Physiological measures in the mussels revealed that leukemia was not present above background levels, but the precursor cells, called transitional cells, were significantly elevated. Condition factors also varied significantly in comparison with a reference site.

Non-professional phagocytosis in fish response to pathogens: roles of stress and toxicant exposure. L.E.J. Lee, R. Donkersgoed and R.M. Slawson. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Phagocytosis is an innate response and most cells are able to perform this function as part of their housekeeping roles. However, in multicellular organisms, specialized phagocytic cells such as macrophages have evolved that perform most of the professional clean-up of debris and ingestion of foreign particles. In aquatic environments, the exposure to pathogens is high and it appears that non-professional phagocytes such as epithelial and fibroblastic cells also aid in the clean-up processes, especially around mucosal surfaces. Cells in culture have been instrumental in elucidating physiological mechanisms and, in this study, phagocytosis was investigated using various fish cell lines derived from mucosal and non-mucosal tissues. Microscopy under normal light, dark field and epi-fluorescence were used to evaluate parameters affecting phagocytic rates. These included presence or absence of serum in culture media, type and size of phagocytic particles, exposure times, density of cultures, hypotonic conditions, cortisol concentration, mechanical stress, and exposure to toxicants. In this study, the modulatory role of aquatic contaminants on phagocytosis and bacterial removal was investigated using several fish cell lines: RTS-11, RTgill-W1, RTG-2 and GFSk-S1 derived from immune, mucosal and non-mucosal tissues of rainbow trout and a skin cell line from goldfish respectively.

Environmental Effects Monitoring - Metal Mining / Étude de suivi deseffets sur l'environnement - mines de métaux

Session co-chairs: E. Gardiner and A.G. Colodey

Effects of water chemistry on copper toxicity to ***Mytilus*** **sp. - implications for saltwater quality criteria in the USA.** W.R. Arnold. Copper Development Association Inc., New York, NY.

Over the past three decades, there have been significant advances in understanding how environmental factors modify the bioavailability of metals, such as copper, in aquatic environments. Several of these advances have led to changes in the U.S. EPA freshwater quality criteria for Cu, to the development of guidelines to indirectly account for modifying factors, and to adjustments of criteria on a site-specific basis. To date, most of these efforts have focused on assessing bioavailability in freshwater systems, but it is reasonable to expect that similar modifying factors exist in marine environments and should be investigated, understood, and used in the environmental management decision-making process. To date, a disproportionate amount of the effort to investigate environmental factors that modify Cu bioavailability has focused on freshwater species. As a result, the only direct change to the saltwater Cu criteria has been its application to the more bioavailable dissolved Cu fraction rather than total Cu. At this time, there remains a need for studies to address modifying factors in estuarine and marine environments and their affects on toxicity to sensitive species used in the development of the national saltwater metal criteria. However, there is existing evidence that water chemistry can strongly influence Cu toxicity in marine waters. It appears we could build on what we have learned in freshwater by testing and applying usable principles to marine waters. Several examples of the effects of water chemistry on Cu speciation and toxicity in marine waters and implications on water quality criteria development and application are discussed.

Modelling metal-gill interactions and metal toxicity to fish: the influence of natural organic matter source. R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Natural organic matter (NOM) binds metals, decreasing the amount of metal binding to fish gills and therefore decreasing metal toxicity to fish. Work in my laboratory has shown that optically darker, more allochthonous (terriginous) NOM binds metals like copper, lead, and aluminum better than does optically lighter, autochthonous-like NOM, as judged by metal binding to gills of rainbow trout (*Oncorhynchus mykiss*) and by metal toxicity to trout. In these experiments, NOM was isolated by reverse osmosis from diverse sources and added at up to 10 mg C/L in the lab. A good index of NOM source is the Specific Absorbance Coefficient at a particular wavelength (e.g., SAC_{340}=$Absorbance_{340}$×2,303÷TOC), as well as excitation-emission matrix characterization of NOM. In contrast, NOM source appears to have minimal influence on the degree of inorganic mercury, silver, and cadmium binding to fish gills. The pattern appears to be that metals which bind more strongly to fish gills (higher metal-gill log K values) than to NOM (lower metal-NOM log K values) are not influenced as much by NOM source. These results have important implications for developing Biotic Ligand Models.

Environmental study at a closed mine where regulated effluent pH limits exceeded natural pH levels. C.K. Russel, P.I. LePage and P.L. Orr. Minnow Environmental Inc., Mississauga, ON.

A study was conducted at the site of a tin mine, which closed in 1992, but continues to discharge treated tailings pond effluent to a naturally acidic stream (pH of about 4.5). A previous study, conducted in 1997 showed greater fish diversity downstream of the mine and attributed this, in large part, to higher pH resulting from the discharge of treated, alkaline effluent (pH of about 8.0). The results of a repeat study, conducted in 2004, will be presented and discussed in terms of any changes that may have occurred since the previous study was conducted. Current policy under the Environmental Effects Monitoring (EEM) requirements of the *Metal Mining Effluent Regulations* (which do not apply to closed mines) define an effect as any mine-related statistical change in the benthic invertebrate or fish communities of the effluent receiving environment compared to those in comparable reference habitats. Although the EEM regulations do not apply to this site, it represents an interesting example of a potential regulatory conundrum: effluent is treated to meet discharge pH limits, but the alteration of pH affects the downstream biological communities, although it is a positive effect.

Contamination of freshwater and marine environments from historical gold mining activities

in Nova Scotia. M.B. Parsons[1], P.K. Smith[2], T.A. Goodwin[2], G.E.M. Hall[3], S. Witch[4], V.P. Palace[5], R. Parrott[1] and A.L. Sangster[4]. [1]Natural Resources Canada, Geological Survey of Canada, Dartmouth, NS; [2]Nova Scotia Department of Natural Resources, Halifax, NS; [3]Natural Resources Canada, Geological Survey of Canada, Ottawa, ON; [4]Department of Earth Science, University of Ottawa, Ottawa, ON; and [5]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

This multidisciplinary project is examining the dispersion, transformation, and fate of metals and metalloids in freshwater and marine environments surrounding abandoned gold mines in Nova Scotia. From 1861 to the mid-1940s, gold was produced from 64 mining districts in the southern part of the province. Most of the gold was recovered using stamp mills and mercury amalgamation, and the tailings were slurried directly into rivers, swamps, lakes, and the ocean. In 2003, samples of tailings, soil, rock, sediment, water, and vegetation were collected at 11 past-producing mines. Chemical analyses of 400 tailings and sediment samples show high concentrations of Hg (<5 μg/kg to 350 mg/kg), as well as other potentially toxic elements (e.g. As, 9 mg/kg to 31 wt.%). The highest Hg concentrations are found near mill structures, reflecting Hg loss during amalgamation and retorting. Water chemistry data indicate that the dissolved concentrations of As are very high at some locations (up to 6,600 μg/L), as compared to background values of generally <25 μg/L. Dissolved Hg levels range from 1-60 ng/L, and show a significant positive correlation with dissolved organic carbon at most sites. Ongoing studies in 2004 are characterizing the distribution, speciation, and bioaccumulation of metal(loid)s in both freshwater and marine systems. A wide variety of methods are being employed including sequential extractions, methylmercury analyses, fish sampling, sediment/water toxicity testing, and multibeam bathymetry mapping. Results from this project will be used to assess the potential risks associated with these sites, and will support better informed land-management decisions.

Use of small forage fish in caged exposures for metal mining Environmental Effects Monitoring (EEM). C. Doebel[1], C.L. Baron[2], K.G. Wautier[2] and V.P. Palace[2]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Environmental Effects Monitoring (EEM) for Canadian metal mines is a program that has been under development since 1993. It has been designed to examine the potential environmental effects of metal mining discharges on fish, fish habitat, and the usability of fisheries resources. Questions surrounding the metal mining EEM program have arisen regarding appropriate methods for conducting fish population surveys, the means through which effects on fish will be monitored. In situations where there are confounding influences, it has been difficult to design survey methods void of bias, representative of existing fish populations, and sensitive enough to determine whether or not effects are occurring. Furthermore, the currently accepted approaches to fish surveys in the EEM program are limited through the fact that fish residency and hence exposure profiles cannot be aptly described. At present, the caged fish approach is not an accepted method in the EEM program for metal mines. In an effort to provide an alternative fish survey method wherein the above listed difficulties are minimized, a series of caging experiments were conducted in northwestern Ontario, Canada. These studies involved caging small-bodied juvenile fish at 2 sites (1 reference, 1 exposure) in an area impacted by gold mining activities for 2-4 weeks. Biological parameters (e.g. growth) and levels of accumulated arsenic species were evaluated. The objective of this research was to refine small-bodied caged fish techniques to allow the incorporation of this method into the Canadian metal mining EEM program.

Harmonization of provincial and federal requirements for environmental effects monitoring at the Eskay Creek Mine, British Columbia.

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Abstract

The Eskay Creek Mine, an underground gold mine located approximately 80 km north of Stewart, British Columbia, has been in operation since 1995. Discharges from the mine (tailings and treated mill effluent) are subject to a provincial discharge permit as well as the federal *Metal Mine Effluent Regulations* (MMER). In fulfillment of the provincial permit requirements, EVS Environment Consultants (EVS) have been conducting annual environmental effects monitoring (EEM) at the mine since 1997 to identify any potential mining-related effects in the receiving environment. Monitoring components have included assessments of water and sediment quality (chemistry and toxicity) and benthic community structure. Results of the monitoring indicate that the waterbodies in the vicinity of the mine are naturally enriched with a few metals and a metalloid (e.g., arsenic, cadmium, iron, and zinc), while the mine discharges have resulted in increasing concentrations of other metals (e.g., antimony), though not to levels of environmental concern. Any observed changes in water and sediment toxicity and benthic invertebrate abundance and diversity appear to be related to natural variability from year to year, rather than to the mine's discharges. We will report on the findings of the annual EEM program between 1997 and 2003, harmonization of the provincial and federal EEM requirements, and on general lessons learned related to EEM programs.

Introduction

Eskay Creek Mine: The is an underground gold mine located about 80 km north of Stewart BC (Fig. 1). Approximately 50% of the ore produced at the mine is processed on-site in a gravity and flotation mill to produce metal concentrates, which are shipped elsewhere for processing. The remaining 50% of the ore is a direct ship product which is also transported off site for final processing. The mine, which has been in operation since 1995, processed 250,000 tonnes of ore and produced over 350,000 ounces of gold in 2003.

The Aquatic Receiving Environment: Resident fish populations are not present in the immediate vicinity of the mine due to the presence of an impassable barrier (Hallam Knight and Homestake, 1993). The Unuk River (primarily downstream of its confluence with Storie Creek, is about 10 km downstream from the mine) supports a variety of fish, including cutthroat trout (*Oncorhynchus clarki*), Dolly Varden char (*Salvelinus malma*), and five species of Pacific salmon (*Oncorhynchus* spp.).

History of EEM Monitoring at the Mine: EVS has conducted annual EEM at the Eskay Creek Mine since 1997, to identify any potential mining related effects on the receiving environment. These studies were initiated in fulfillment of the requirements a provincial permit issued by the British Columbia Ministry of Water, Land and Air Protection (MWLAP), which allows the authorized discharges of minewater to Ketchum Creek, and waste rock and tailings to two nearby lakes, Albino Lake (AL) and Tom MacKay Lake (TM1) (Fig. 2).

Under the provincial permit, the Eskay Creek Mine EEM program has undergone annual review and revision to ensure that the objectives of the program are being met. Since the inception of the EEM program, several different parameters have been monitored as part of the annual EEM and baseline monitoring programs (Table 1). Each of these approaches has been assessed on an ongoing basis to determine the most effective site-specific methods. If a particular monitoring component consistently demonstrated the absence of mining-related effects, the frequency of monitoring was reduced or the component removed from the program altogether. Conversely, if new issues arose, the EEM program was modified accordingly.

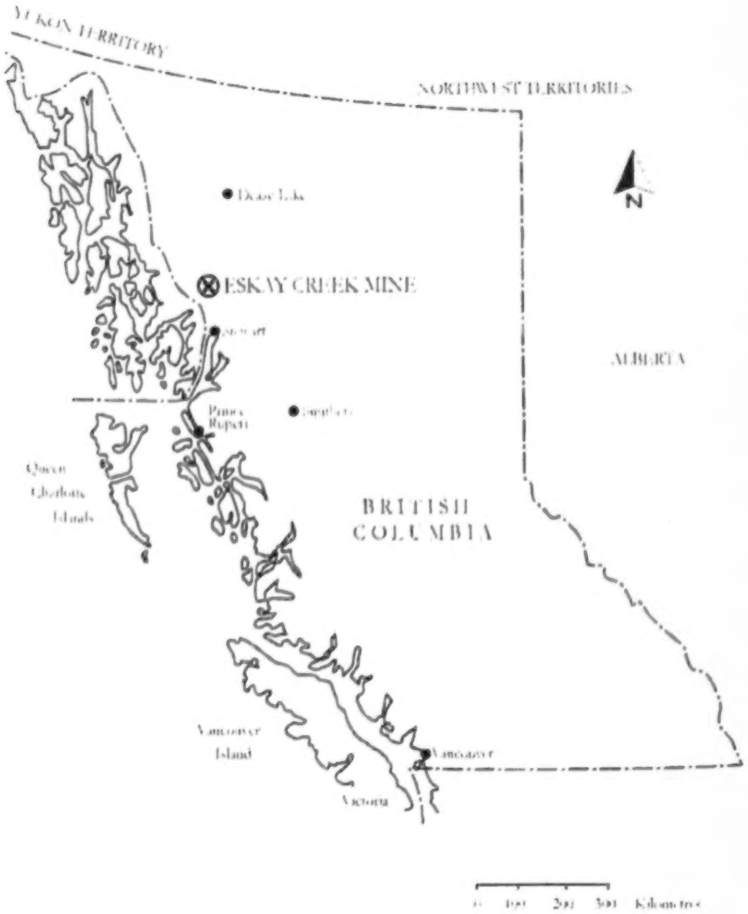
YUKON TERRITORY
NORTHWEST TERRITORIES
N
Dease Lake
ESKAY CREEK MINE
Stewart
ALBERTA
Prince Rupert
Smithers
Queen Charlotte Islands
BRITISH COLUMBIA
Vancouver Island
Vancouver
Victoria
0 100 200 300 Kilometres

Fig. 1. Location of the Eskay Creek Mine.

Overview of EEM Results

The Eskay Creek area is highly mineralized and therefore both water and sediments have shown elevated metals concentrations (compared to applicable guidelines) at reference sites as well as at downstream exposure stations (EVS 2004). Discharges to the two tailings impoundments and Ketchum Creek (mine processing effluent) appear to have resulted in increasing concentrations of, for instance, antimony, however, results of water and sediment toxicity testing and benthic community structure indicate that the discharges are not having an effect in the receiving environment.

The results of taxonomic identification and enumeration of benthic invertebrates have found some differences between exposed and reference stations; however, these differences are more likely due to intrinsic habitat differences (i.e., elevation, gradient, substrate characteristics) between the sites than mine-related discharges (EVS 2004). This conclusion is supported by the fact that sediment toxicity testing using *Chironomus tentans* consistently showed limited toxicity. In fact, survival and growth at the station with the highest metals concentrations (Station AL) have remained relatively high throughout sediment toxicity testing.

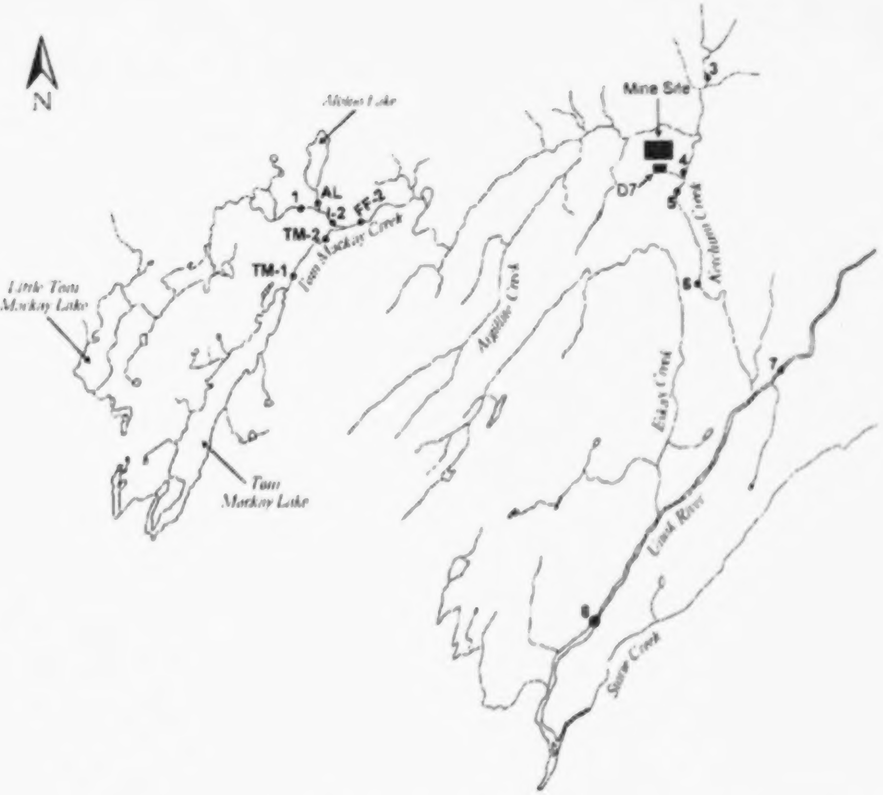

Fig. 2. Location of drainages and sampling stations for the Eskay Creek Mine EEM program.

Toxicity tests (7-day *Ceriodaphnia dubia* partial life cycle test, 72-h *Selenastrum capricornutum* algal growth inhibition test, and rainbow trout (*Oncorhynchus mykiss*]) acute lethality have been conducted at Stations 1 (reference), AL, TM-1, and downstream of Tom Mackay Lake discharge (TM-2). Overall, the results have indicated a very low frequency (e.g., LC_{50}'s for *O. mykiss* and *C. dubia* are almost always >100% v/v) and inconsistent pattern of toxicity at all stations including the reference location, Station 1.

Caged bivalve studies were conducted at Eskay in 1999 and 2000, to assess effects of effluent exposure on metal uptake and growth (EVS 2000, 2001). Results indicated that growth (shell length and soft tissue weight) of the freshwater mollusc *Margaritifera falcata* was not affected after eight weeks of exposure to effluents. Increases in tissue weight were observed at both locations, confirming that the molluscs continued to feed and grow during the exposure. The tissues were also analysed for total metals and methyl mercury. Mean total mercury concentrations were <0.06 mg/kg, which was well below the provincial guideline of 0.5 mg/kg.

Comparison of Provincial and Federal EEM Requirements

The mine became subject to the federal Metal Mining Effluent Regulation (MMER) in June, 2002, and will be conducting its first EEM program under the MMER in the fall of 2005. Table 1 shows a comparison of the components currently or formerly in use for the provincial permit, and the components required by the federal EEM program. The provincial EEM program currently entails water chemistry and toxicity, sediment chemistry and benthos, and is generally consistent with the federal EEM requirements, with a few exceptions including monitoring frequency, analytical parameters for water (i.e., the provincial permit does not require analysis of radium) and sediment chemistry (i.e., the provincial permit requires metals analysis) chemistry, and test species for water toxicity (i.e., *Lemna minor* was not used under the provincial permit, and *Ceriodaphnia dubia* was used instead of *Daphnia magna*).

Table 1. Eskay Creek Mine - summary of program components for existing EEM related to provincial permit and requirements for federal MMER.

PROGRAM COMPONENTS	EXISTING PROVINCIAL EEM[1]	FEDERAL EEM REQUIREMENTS
Water chemistry	Yes	Yes (additional parameters required)
Water toxicity	Yes	Yes
Metal speciation	Yes	No
Sediment chemistry	Yes	Yes (fewer required parameters)
Sediment toxicity	Yes (intermittent frequency)	No
Benthos	Yes (riffle, depositional, lake)	Yes
Drift fauna	Yes	No
Zooplankton	Yes	No
Periphyton (taxonomy and chlorophyll)	Yes	No
Fish Populations and Tissues	No fish (bivalve accumulation attempted)	No fish (alternative required)

Notes:

[1] Several different components have been assessed for use at Eskay Creek Mine since the inception of EEM in 1997, but are no longer used on an ongoing basis (e.g., periphyton, zooplankton community).

The MMER requires fish population studies if the effluent concentration in water is >1% within 250 m of the final discharge point. Fish tissue studies are only required if the total mercury concentration in the effluent is ≥0.10 μg/L. Based on these conditions, both studies are technically required at the Eskay Creek Mine. However, at Eskay Creek, standard fish surveys cannot be conducted as there are no resident fish populations. An impassable barrier at the outlet of Ketchum Creek prevents fish access to the watercourses in the vicinity of the mine (Hallam Knight and Homestake 1993). As an alternative to direct assessment of fish, a caged bivalve study has been used in Ketchum Creek (Section 1.4).

Lessons Learned

The MMER offer many benefits to the environment, regulatory agencies and the mining community. Standard requirements for EEM help ensure that all mines of a given size assess the potential for mining-related effects in the receiving environment. These standard requirements als provide environmental agencies with common tools, regional consistency, and the ability to compar potential mine-related effects across the country. For the mining industry, the regulation provides lega certainty that mine discharges are permissible, and clearly defines the goalposts. It also gives mine new tools to show members of the community and potential opponents to mining that mines ca operate in an environmentally sustainable manner.

Unfortunately, for some sites, the standard requirements may result in the use of tools o application of measuring criteria that are not environmentally relevant, and expenditure of resource that do not provide useful information in terms of detecting mine-related effects. Some examples fro the Eskay Creek Mine include the following:

(a) *Lemna minor* and *Selenastrum capricornutum* are currently included in the twice-yearl sublethal toxicity testing required by the MMER. Under the provincial permit, *L. minor* was no initially selected as a test organism because it was determined not to be biologically relevan macrophytes have not been found in the oligotrophic waters of the creeks surrounding the mine. *S capricornutum* was originally included in the suite of test organisms used, however, it was found t be less sensitive than *C. dubia* and therefore discontinued.

(b): The MMER require water quality monitoring in the "exposure areas surrounding the point o entry of effluent into water." Based on discussions with Environment Canada, water qualit monitoring stations have been established at three locations (Stations I-2, FF-2, and 5; Fig. 2 However, in the case of the Eskay Creek mine, it is difficult to separate the discharges from th tailings impoundments from the receiving environment because the outlet creeks are technically th effluent discharges. Water from Tom MacKay Lake comprises 25% (4:1 dilution) of the volume i Tom MacKay Creek at its confluence with Ketchum Creek. Therefore, Ketchum Creek could b considered the exposure area for discharges from both the tailings impoundments as well as th minewater discharge. Furthermore, Ketchum Creek is the final discharge point for all mine-relate discharges to the Unuk River. Therefore, a suggested alternative is to conduct water qualit monitoring in Ketchum Creek only, unless effluent monitoring indicates that parameters of concer have increased to levels of concern.

As discussed above, fish are absent from Ketchum Creek and Tom MacKay Creek. The MME guidance document provides alternative approaches for assessing the potential for bioaccumulati of metals of concern, including extended early life stage (ELS) sublethal toxicity testing and cage bivalves. In 2005, the first EEM cycle under the MMER will include a modified (i.e., off-channe caged bivalve study. However, the relevance of a caged bivalve study to the Eskay Creek Mine uncertain. *In situ* caged bivalve studies were conducted in 1999 and 2000. Challenges in setting t studies up included getting applicable approvals (i.e., transplant permits) for using non-native specie and loss of cages due to flashflooding in Ketchum Creek. The studies showed that there we statistical differences in metals concentrations in mussels from Station 3 (reference) and Station however, it is difficult to assess the applicability of these observations to the potential f bioaccumulation in fish populations downstream for several reasons including: (1) bioaccumulati of metals by bivalves does not necessarily reflect the body burden of fish food organisms present Ketchum Creek and therefore the potential for bioaccumulation in fish; and (2) an assessment of dr fauna suggested that Ketchum Creek is unlikely to provide a significant source of food organisms f fish in the Unuk River (EVS, 2000).

Summary

The Eskay Creek Mine has been conducting environmental effects monitoring as required by a provincial discharge permit since 1997, and has evolved over time, to focus on biologically relevant monitoring tools. The provincial EEM program currently entails water chemistry and toxicity, sediment chemistry and benthos, and is generally consistent with the federal EEM requirements. While the MMER offer many benefits to the mining industry, including clearly defined targets, some of the standard requirements may not be suitable or useful for all mines. More flexibility in the MMER is required to allow it to fully meet its intended goals including environmental protection, reasonable uniformity, and cost effectiveness.

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Development and implementation of a *Metal Mining Effluent Regulations* - Environmental Effects Monitoring (EEM) program at a uranium mine in northern Saskatchewan. M.A. Balych and K.D. England. Cameco Corporation, Saskatoon, SK.

The Rabbit Lake Operation releases treated effluent from a uranium mining/milling operation to a receiving ecosystem which drains into one of Saskatchewan's largest lakes. This paper summarizes the development and implementation of the *Metal Mining Effluent Regulations* - Environmental Effects Monitoring program at Cameco's Rabbit Lake uranium mine/mill in northern Saskatchewan.

Setting ecological and risk-based corporate performance targets for developing tailings-management plans. J.M. Mckernan[1] and D.F. Stewart[2]. [1]Tetres Consultants Inc., Winnipeg, MB; and [2]DFS Consulting, Winnipeg, MB.

Developing a long-term Tailings-Management Plan for an acid-generating tailings area is a major challenge for a private-sector corporation. Accurate definitions of current ecosystem health, current environmental impacts and the current state of ecosystem risk are important for defining the practical extent of environmental protection and/or improvement in ecosystem health to be sought be implementation of a Tailings-Management Plan. Determining the current state of ecosystem health, and the current extent of environmental impacts, depends upon accurate determinations of contaminant transport, mixing dynamics in a receiving watercourse, simultaneous measurements of water chemistry and biotic exposure, and attempts to achieve precise correlation of tissue- and water-chemistry sampling data. A site-specific approach to defining practical corporate-performance targets for a tailings-management plan will be discussed, emphasizing the potential value of such techniques as packed-column metal- attenuation trials, EEM -based environmental monitoring, groundwater- flow- and river-mixing-zone modeling, in situ caged-mussels exposure trials and toxicological evaluations, and Ecological Risk Assessment.

Summary of 2003 metal mining effluent characterization, toxicity, and water quality monitoring data in Pacific and Northern Region. S. Blenkinsopp[1], J-M. Ferone[1], P. Siwik[1] and G. Groskopf[2]. [1]Environment Canada, Environmental Protection Branch, Edmonton, AB; and [2]Environment Canada, Environmental Protection Branch, Regina, SK.

Under the *Metal Mining Effluent Regulations* (MMER), metal mines discharging into the aquatic environment are required to conduct effluent characterization and acute lethality testing on each final discharge point. In addition, mines are required to monitor water quality in a reference and exposure area around each final discharge point, as well as conduct sublethal toxicity testing of effluent from the final discharge point with the most potential for adverse impact on the aquatic environment. The first set of annual data required under the MMER was received in early 2004 for the calendar year 2003. This poster will present a summary of the data submitted by 23 regional mines located in Manitoba, Saskatchewan, the Northwest Territories, and Nunavut. Trends and issues will be identified and discussed.

The Greater Northern Ontario Mining Effects Study - Reference Site Analysis. M.F. Bowman[1] C. Brereton[2], W. Keller[3] and K.M. Somers[4]. [1]Department of Zoology, University of Toronto, Toronto, ON; [2]Department of Biology, Laurentian University, Sudbury, ON: [3]Cooperative Freshwater Ecology Unit, Ontario Ministry of Environment, Sudbury, ON; and [4]Dorset Environmental Science Centre, Ontario Ministry of Environment, Dorset, ON.

The Reference Condition Approach (RCA – see Fig.1) is identified as an alternative assessment method under the metal mining aquatic Environmental Effects Monitoring (EEM) program. This study is a collaboration between industry, government and university partners (see Acknowledgements) to evaluate the RCA using benthos in northern Ontario.

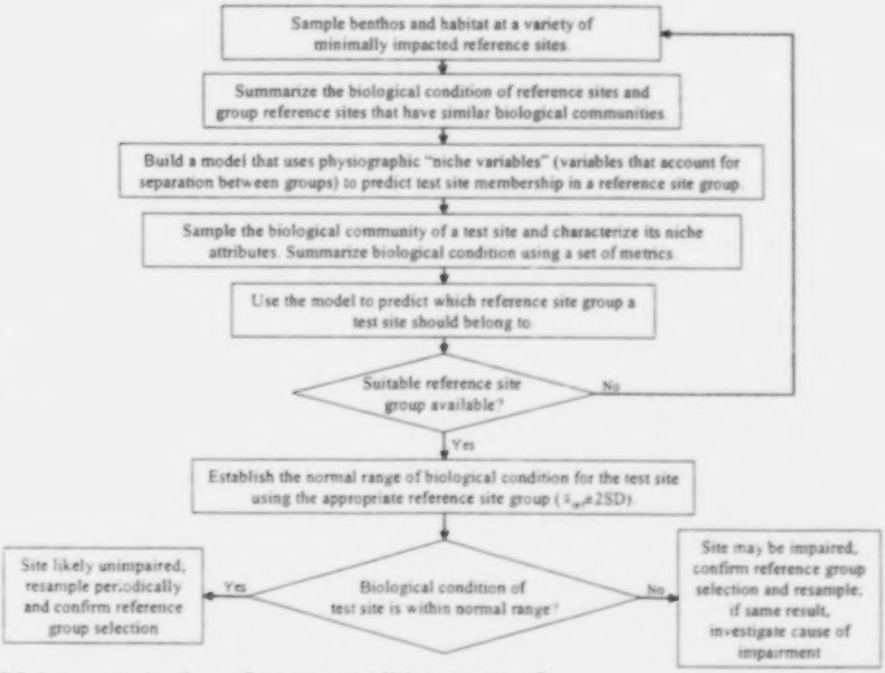
Sample benthos and habitat at a variety of minimally impacted reference sites.
Summarize the biological condition of reference sites and group reference sites that have similar biological communities.
Build a model that uses physiographic "niche variables" (variables that account for separation between groups) to predict test site membership in a reference site group.
Sample the biological community of a test site and characterize its niche attributes. Summarize biological condition using a set of metrics.
Use the model to predict which reference site group a test site should belong to.
Suitable reference site group available?
No
Yes
Establish the normal range of biological condition for the test site using the appropriate reference site group ($\bar{x}_{ref}$±2SD).
Biological condition of test site is within normal range?
Yes
Site likely unimpaired; resample periodically and confirm reference group selection
No
Site may be impaired, confirm reference group selection and resample; if same result, investigate cause of impairment

Fig. 1. Major steps in the reference condition approach

In the fall of 2003, benthic invertebrates were collected from minimally impacted reference areas in 58 streams and the littoral zones (<1 m) of 94 lakes in the vicinity of Red Lake, Hemlo, Sudbury, and Timmins (Fig. 2). Benthos were collected using a 500 μm-mesh net and standardized travelling kick protocol. Fixed counts of 300 animals were subsequently removed from the preserved samples in the lab using a Marchant box and microscope and were generally identified to the Family taxonomic level.

One of the first steps in RCA is to group minimally impacted sites and identify biologically similar community types (Fig. 1; for a detailed description of the RCA, see Bailey *et al.* 2004). Because there is relatively little information on benthic communities in northern Ontario, we had no *a priori* grounds for separating lakes and streams or the four locations. As a result, this study focused on testing lake-versus-stream and location grouping hypotheses.

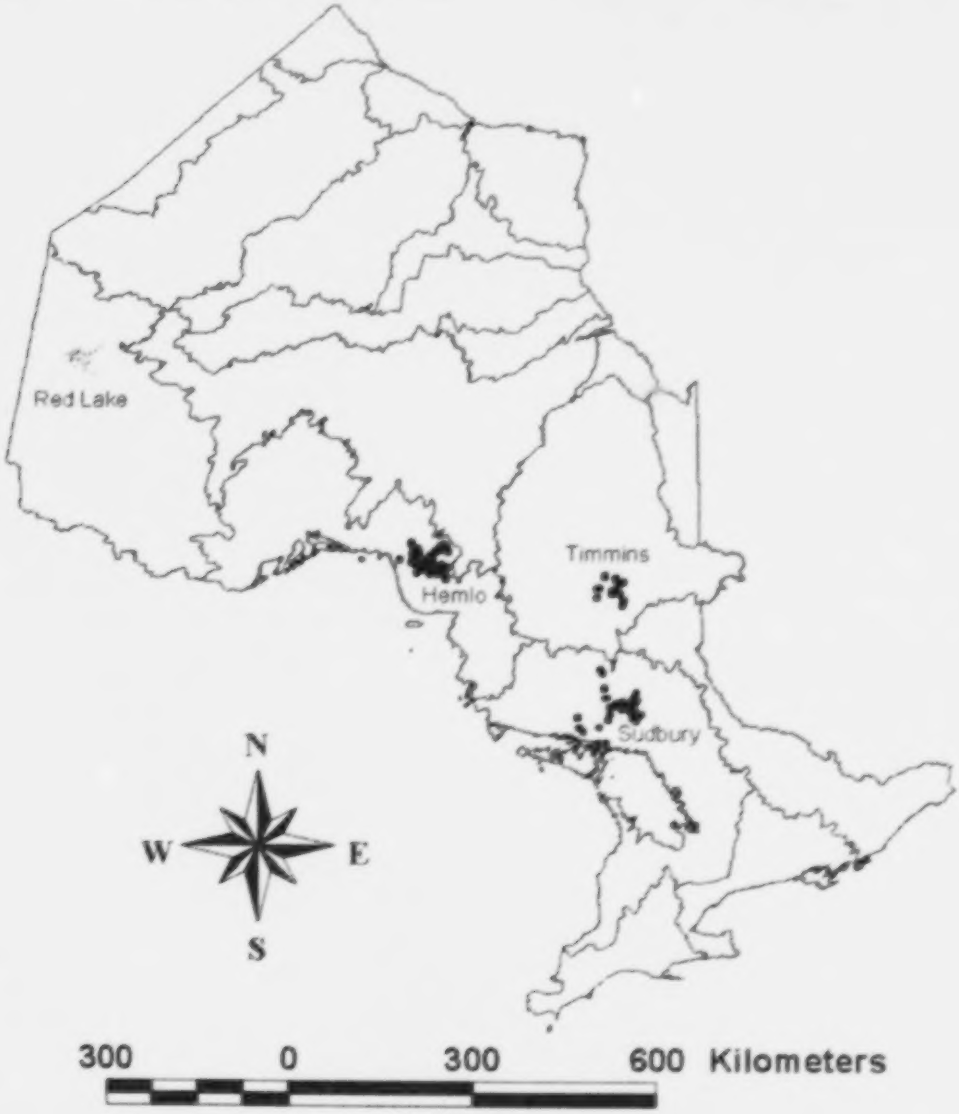

Fig. 2. The greater northern Ontario sampling sites.

To identify clusters of sites representing different community types, sites were grouped using common ordination (correspondence analysis [CA] and NMDS) and clustering (i.e., UPGMA, Ward's, TWINSPAN, K-means) methods (Fig. 3). Both abundance data with the Bray-Curtis (BC) distance and presence-absence (p/a) data with the Jaccard (J) coefficient were used in clustering. The resultant trees (Fig. 4) and ordinations (Fig. 5) were evaluated using Mantel tests correlating cophenetic (tree) or ordination distances and hypothesis matrices.

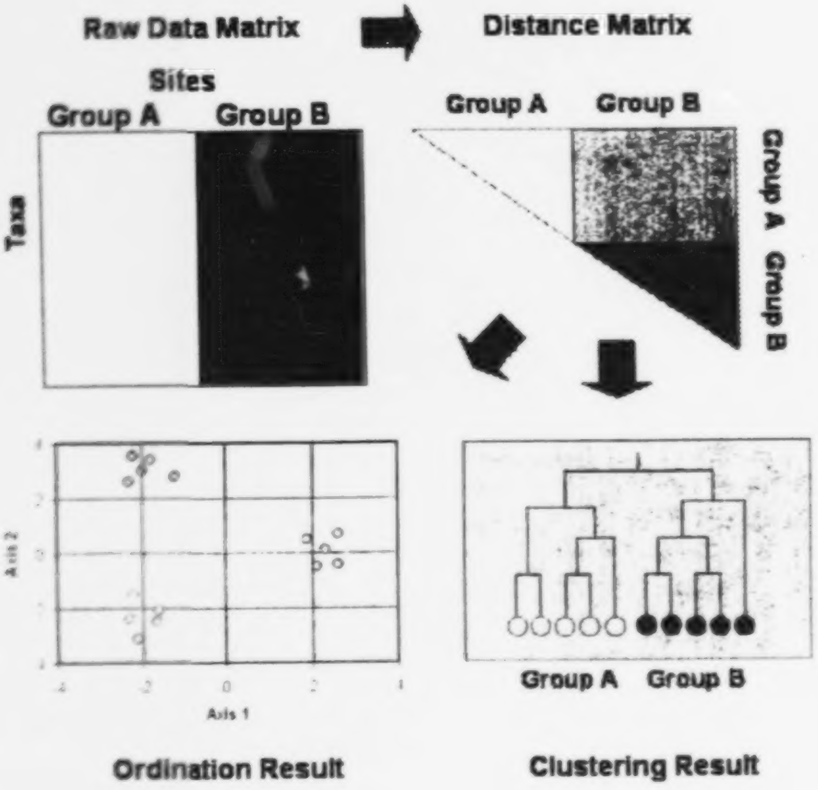

Fig. 3. Steps for grouping sites.

The Mantel test is a type of nonparametric MANOVA with probabilities generated by a randomization algorithm (Sokal 1979, Jackson and Somers 1989). Mantel tests are generally based on simple correlations between two site-by-site distance matrices. We used Mantel tests to evaluate whether sites were grouped by habitat (lake versus stream) or location and examined consensus among grouping methods. Hypothesis matrices were constructed by calculating between-site Euclidean distances using a dummy variable that classifies sites by habitat type or location (e.g., see Somers and Green 1993).

Our results (see Table 1) indicate highly significant community groupings by habitat (i.e., lake versus stream) for all clustering and ordination methods, but considerably less evidence of a location effect. Groupings based on p/a data were often more highly correlated with habitat (and location) indicating the importance of taxonomic richness in group separation; this finding was unexpected given the use of Family-level taxonomic resolution.

In the next step of the RCA, construction of niche-based predictive models (see Fig. 1) will combine data from the 4 locations, but lake and stream communities will be modelled separately.

Acknowledgements – This study was possible because of collaboration among individuals and funding from Inco, Goldcorp, Newmont Canada, Placer Dome, Williams Op. Corp., Environment Canada,

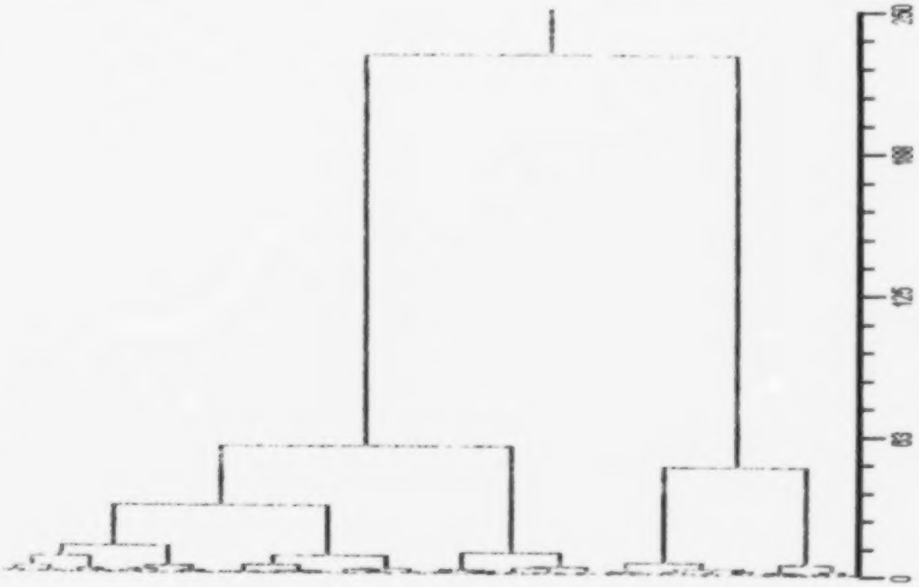

Fig. 4. Example clustering solution from NTSYS (Rohlf 2000).

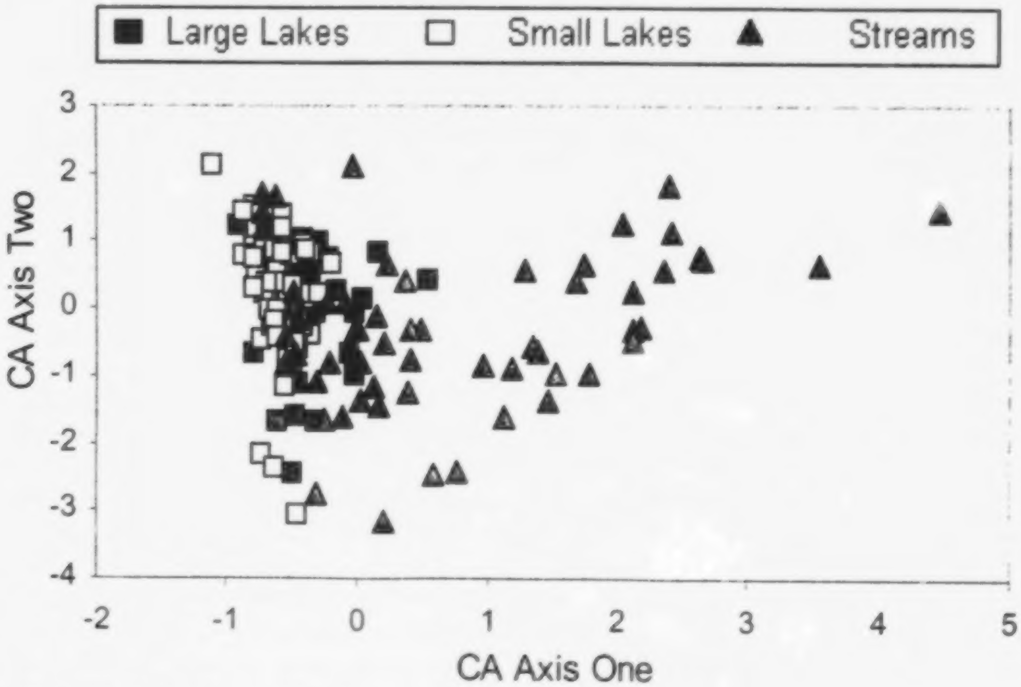

Fig. 5. Example ordination solution

Table 1. Results of the Mantel tests evaluating groupings of sites based on different clustering and ordination methods using NTSYS (Rohlf 2000) and PopTools (Hood 2004).

Method	Mantel test results			
	Lake versus stream		Location	
	r	P	r	P
UPGMA – BC	0.16	0.001	0.01	0.567
UPGMA – J	0.52	0.001	0.06	0.076
Ward's – BC	0.12	0.001	0.01	0.465
Ward's – J	0.53	0.001	0.04	0.083
TWINSPAN	0.37	0.001	0.10	0.006
K-Means – abundance	0.52	0.001	0.03	0.165
K-Means – p/a	0.66	0.001	0.02	0.226
CA – abundance	0.37	0.001	0.12	0.009
CA – p/a	0.34	0.001	0.07	0.065
NMDS – abundance	0.11	0.001	0.03	0.678
NMDS – p/a	0.32	0.001	0.19	0.001

Acadia University, and the authors' institutions. Fig. 1 was originally prepared by Chris Jones based on discussions with Trefor Reynoldson; Fig. 3 was first drafted by Bruce Kilgour; we thank them for the loan of their draft figures.

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Influence of natural organic matter quality on aluminum binding to gills of rainbow trout. A.R. Winter, T.A.E. Fish and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Aluminum (Al) readily accumulates on the gills of fish. The addition of natural organic matter (NOM) decreases Al binding to fish gills. It has been shown by us that the source of NOM is usually important when investigating metal complexation and metal toxicity to fish. We added NOM concentrations of 1.2, 2.5 and 5.0 mg C/L from ten different sources to 3 μM Al in ion-poor water at pH 6.5. Rainbow trout (*Oncorhynchus mykiss*; 1 g) were exposed to these solutions for 3 h. Measured gill Al showed that optically dark organic matter from terrigenous sources (allochthonous NOM) complexes Al to a greater degree than does optically light (autochthonous) organic matter. For both types of NOM, the degree of metal complexation increased with NOM concentration. These differences observed in NOM metal-binding quality are important to consider when investigating Al toxicity in the aquatic environment.

The effects of natural organic matter source on silver uptake by gills of rainbow trout. T.A.E. Fish, A.R. Winter and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Silver, like other metals, is less available for uptake by gills of rainbow trout when silver is bound to organic matter. This study investigated whether natural organic matter (NOM) samples from various sources have distinct strengths of binding to Ag that result in different amounts of Ag accumulation by trout gills. Ten different NOM sources were collected using a portable reverse osmosis unit, and were tested using concentrations of NOM from 2-10 mg C/L total organic carbon. Juvenile rainbow trout (*Oncorhynchus mykiss*, 1 g) were exposed to 0.25 μM Ag in ion-poor water at a pH ~7 in the presence of the NOM. Ag accumulation by the gills was analyzed by graphite furnace atomic absorption spectrophotometry. As judged by Ag accumulation by fish gills, NOM from different sources does not differentially bind Ag. As well, chloride complexation of Ag is a complicating factor that also decreased Ag accumulation by trout gills. Using 5-20 mg C/L total organic carbon of marsh NOM and fish food NOM to minimize the chloride effect seen previously, a Ag toxicity test using 0.5 μM Ag in ion-poor water was run. Initial results suggest that optically dark (marsh) NOM does not provide a greater protective effect than optically light (food) NOM, again suggesting that different NOM sources do not have distinct strengths of binding to Ag.

The bioaccumulation of select metals and metalloids in a smelter impacted aquatic ecosystem. L. Desbiens[1], G.A. Spiers[1] and J. Gunn[2]. [1]Centre for Environmental Monitoring, Laurentian University, Sudbury, ON; and [2]Department of Biology, Laurentian University, Sudbury, ON.

In recent years the trophic transfer of metals along aquatic food chains has been recognized as an important issue in the study of water quality. The most direct way of quantifying bioavailable contaminants is to measure bioaccumulation by organisms. Even though bioaccumulation of metals by aquatic organisms is a reasonably well documented field of research, the amount of information regarding many of the trace elements and their effects on freshwater ecosystems is still limited. Sudbury, ON, developed as an industrial center focused on the smelting and refining of the natural ores of nickel and copper. As a result of these industrial activities, the surrounding landscapes and habitats have suffered environmental insult from gaseous and particulate emissions emanating from the smelters. Ramsey Lake, located in Sudbury, ON, is a unique lake in that it serves both as a major recreational resource for the community and the drinking water source for approximately 50,000 citizens. This research project is investigating the transfer and fate of the following metals: Co, Ni, Cu, Zn, Pb and metalloids: As, Se, Th and antimony in the aquatic food chain of Ramsey Lake. Sediment, water, phytoplankton, zooplankton and fish are being collected and analyzed to determine if bioaccumulation and/or biomagnification is occurring between these two groups of elements.

Use of surface water, pore water and sediment bioassays with *Hyalella azteca* to identify the cause of benthic impacts at a Saskatchewan uranium mine/mill. E.L. Robertson and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Past monitoring has noted benthic community structure impairment downstream of the Key Lake uranium mine/mill discharge site in northern Saskatchewan, Canada. This research was aimed at identifying the cause(s) of this impact. Preliminary work used a Sediment Quality Triad approach to verify the presence and extent of benthic community impairment at a series of downstream sites, as well as develop hypotheses on what stressor(s) could be responsible for the observed effects. Results confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface water, pore water and sediment chemistry. Hypotheses generated from these data suggested ammonia and arsenic as possible causes of toxicity. This summer, surface water, pore water and whole-sediment bioassays were conducted using laboratory-cultured juvenile *Hyalella azteca* as part of a more focused investigation. An *in-situ* experiment with caged amphipods was also performed. Surface water and pore water bioassays were 96-h in duration with daily renewal of test solutions. Whole-sediment tests were 10-day in duration with automated overlying water renewal twice daily. Endpoints included survival, growth and metal bioaccumulation. This approach was used to help determine which environmental compartment(s) could be responsible for the benthic community impairment observed *in-situ*. Results will be presented on the associations among *H. azteca* mortality, growth, and metal bioaccumulation in relation to contaminant concentrations in surface water, pore water and sediment.

Serendipity in Aquatic Sciences / Les découvertes fortuites en sciences aquatiques

Session co-chairs: J. Hellou and P.M. Chapman

Serendipity is the future of aquatic ecotoxicology. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Scientists do not typically believe that they depend on serendipity to advance their science, because they generally equate this term with luck. However, serendipity is not luck, but rather results from an ability not only to recognize a new discovery, but also to do so within an appropriate multi-disciplinary context. Major advances in aquatic ecotoxicology will not be made by those with a narrow viewpoint, but rather by those with the widest possible viewpoint. At a minimum, aquatic ecotoxicologists must have good knowledge of both toxicology and ecology. For example, major recent advances have occurred because researchers could relate biota behaviour to their response to stressors. Ecotoxicologists should also have good knowledge of environmental chemistry, to understand the fate and effects of contaminants not just in their model systems, but also in the real environment - to be able to make and test predictions. Knowledge of other disciplines is also highly desirable. For instance, aquatic ecotoxicologists with knowledge of hydrology have been able to explain apparently anomalous results between laboratory and field toxicity due to this uncommon route of exposure. There have been major advances in our understanding of the significance of adaptation to contaminants because researchers had the appropriate knowledge of genetics. Multi-disciplinary teams are far less useful if each individual only knows their own discipline. Current emphasis on holistic approaches such as risk assessment, weight of evidence, watershed-level assessment, demand both multi-disciplinary knowledge and context. Without these, serendipity is most unlikely, as are major advances in aquatic ecotoxicology.

Aquatic toxicity of petroleum: an evolving paradigm. J.M. Neff. Batelle Memorial Institute, Duxbury, MA.

Serendipity has been an important element in our evolving understanding of the causes of the toxicity of spilled petroleum to freshwater and marine organisms. Crude and refined oils are extremely complex mixtures of thousands of organic chemicals, mainly saturated and aromatic hydrocarbons. The composition and toxicity of oil changes rapidly through various weathering processes after a release to an aquatic ecosystem. Studies in our laboratories in the early 1970s revealed a direct correlation between the concentration of total aromatic hydrocarbons, in fresh, unweathered crude and refined oils and the toxicity of dispersions and water-soluble fractions (WSF) of the oil to marine animals. For many years, it was concluded that dissolved aromatic hydrocarbons are the dominant toxicants in spilled oil. However, recent studies with weathered crude oils and diesel fuels have revealed that the monocyclic aromatic hydrocarbons usually are lost from the oil so rapidly that they do not contribute significantly to the toxicity of the oil in the water column. The contribution of dissolved polycyclic aromatic hydrocarbons (PAH) to the aquatic toxicity of oil decreases as the oil weathers. Polar oxidation products of saturated and aromatic hydrocarbons, produced by microbial metabolism and photo-oxidation, may contribute to oil toxicity as the oil weathers in the environment. PAH and hydrocarbon degradation products also may partition directly from minute oil droplets, produced by physical dispersion of weathered oil, into filter-feeders (bivalve mollusks) that ingest them and demersal/benthic eggs and larvae that come in contact with oil droplets. The toxicity of oil in sediments is related to the biological accessibility of low molecular weight PAH and hydrocarbon degradation products that partition from the oil phase into pore water or directly into the tissues of marine organisms living in the sediments. The biological accessibility of hydrocarbons, including PAH, from oil in sediments decreases as the oil weathers, decreasing oil toxicity.

Marine natural products, a valuable source of chemical diversity for drug discovery research (sometimes it is better to be lucky than it is to be good). K.R. Gustafson. National Cancer Institute, Frederick, MD.

Marine algae, invertebrate animals and microbial isolates are a rich source of structurally unique secondary metabolites. In addition to new and unusual structural features, many of these compounds also exhibit potent biological activities. The U.S. National Cancer Institute (NCI) has an extensive natural product lead discovery program to identify new compounds with antitumor or anti-HIV properties, and much of this effort is focused specifically on marine samples. The combination of high throughput screening of natural product extracts, with increasingly sensitive spectral characterization techniques, has proven very effective for new lead compound discovery and structural elucidation. An overview of the NCI natural products program and some recent results from these efforts will be presented. Marine natural products constitute a valuable resource for bioassay-guided isolations, however the ultimate success of these studies is often dependent upon factors that we can not directly control.

Potential catastrophic legacy: the case of the missing chlorine tankers. S.M. Bard and A. Edwards. Faculty of Science, Dalhousie University, Halifax, NS.

On the dark and stormy night of February 19, 1975, four tank cars filled with liquid chlorine were swept off a barge and lost at sea. The barge was being towed by a Seaspan tug boat in Malaspina Strait along the British Columbia coast enroute to the MacMillan-Bloedel Powell River pulp mill. The exact location of the sunken tankers has never been determined. Department of Fisheries and Oceans (DFO) risk assessment studies carried out in 1977 suggest that the tankers are likely located at a depth greater than 30 m where any leaking chlorine would remain in the liquid phase. The tank valve manufacturer predicted that the valves would corrode and leak chlorine into the surrounding benthic environment by the mid-1980s. DFO has predicted that minimal leaking would destroy benthic habitat in the immediate area surrounding the tankers. We have examined the risk to the marine environment and local human population were a catastrophic rupture of the tanks to occur, such as from an earthquake. We will present a model of the physical extent and biological effects of the large scale release of chlorine into the marine environment and atmosphere. We will discuss who would be ethically and legally responsible for the potential acute and chronic environmental damages and human health effects from such a release. Recovery strategies will be proposed.

The role of serendipity in interdisciplinary environmental research. M.B. Parsons. Natural Resources Canada, Geological Survey of Canada, Dartmouth, NS.

Over the past several decades, dramatic advances have been made in many different fields of environmental research concerning interactions between humans and the geosphere, biosphere, hydrosphere, and atmosphere. Increased understanding of the complex linkages between these systems has highlighted the need for interdisciplinary research, and has led to the development of 'Earth Systems' programs in many universities to help students gain a solid grounding in various scientific and non-scientific (e.g., law, economics) disciplines. Mastering these varied fields is a daunting task for students and beginning professionals, and the idea of making new, important scientific discoveries purely by accident can seem like a very remote possibility. However, it is the author's belief that serendipitous scientific discoveries are not out of the realm of possibility for young researchers, especially those who are willing to engage in open-minded conversations with scientists from other disciplines. Having access to cutting-edge research tools can certainly help, but the willingness to seriously consider and follow-up on alternate working hypotheses is probably far more important. In this presentation, the author will share his personal views on the role of serendipity in advancing the field of environmental geochemistry, and the upsides and downsides of conducting research in academia, the private sector, and the federal government.

Maximizing serendipitous scientific discoveries. K. Hedley. Environment Canada, National Water Research Institute, Gatineau, QC.

There is little doubt that serendipity, the natural gift of making useful discoveries by accident, has played a huge role in some of the world's most significant scientific discoveries to date. The list of serendipitous discoveries includes the microwave oven, post-it notes, penicillin, immunization, plastics, and most recently, Viagra®. What is perhaps a subject of more serious debate is how governments can use their funding mechanisms to maximize the chances that a major break-through will result from the research. Some renown scientists, including Nobel Laureate Arthur Kronberg, have argued that basic research, defined generally as research with the objective of furthering knowledge out of curiosity, as opposed to applied research, research with an objective of solving a problem and having a use in the end, must be funded adequately in order to maximize probability of big discoveries. However, a review of some of the serendipitous discoveries in science show that discoveries appear to be more often made when scientists are attempting to produce one result, but something else occurs, and the scientist as able to recognise this as not a failed experiment, but a new opportunity. What we need in increase is the sagacity of scientists - being in the right place at the right time and having the ability to realise the significance of what is happening.

Serendipity and environmental science: is there a place for serendipity in science? J. Hellou[1], P.M. Chapman[2], J.M. Neff[3], K.R. Gustafson[4], S. Bard[5], M.B. Parsons[6] and K. Hedley[7]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; Scotia; [2]EVS Environment Consultants, North Vancouver, BC; [3]Batelle Memorial Institute, Duxbury, MA; [4]National Cancer Institute, Frederick, MD; [5]Faculty of Science, Dalhhousie University, Halifax, NS; [6]Natural Resources Canada, Geological Survey of Canada, Dartmouth, NS; and [7]Environment Canada, National Water Research Institute, Gatineau, QC.

This extended abstract is based on the platform session named above that took place on the last afternoon of the workshop. The first two authors were co-chairs of this session and sent a short description when inviting their speakers to submit abstracts, to stimulate the direction of the presentations: (1) has science changed for the better or worse over the past few decades? (2) Will the next generation laugh at us or envy us? (3) What is needed to do good science?

The consensus was that science is different now than it was in the past, but can not be classified as either better or worse. The next generation will do what it will do; all we can do is the best we can.

The primary focus of the presentations was on what was needed to do good science. Clearly, there is a need to go beyond one's comfort zone, to challenge oneself and one's ideas constantly, and to listen to and accept criticism when merited. We are scientists, after all, and learn by making mistakes. Advancing our collective knowledge of the environment involves taking risks; we must not play it safe.

A good example of the development of science is provided by the evolving paradigm regarding the aquatic toxicity of petroleum. Work done some 30 years ago, because it was technically defensible, still forms a solid foundation for work being done today (Anderson *et al.* 1974). This early work demonstrated the importance of aromatic hydrocarbons in the aquatic toxicity of fresh petroleum; recent work, including other presentations at the 2004 Aquatic Toxicity Workshop, documents the importance of oil droplets and degradation products in the chronic toxicity of weathered oil.

As the title of this article implies the emphasis in this session was the role of serendipity in developing science. In environmental science, it is definitely not true that "blind luck rules", however by being open minded, we can end up by "making our own luck" and use this luck to improve the overall science.

It has been said that "... it is better to be lucky than it is to be good". In science it is better to be lucky as well as good. This is illustrated by ongoing research that aims to discover naturally occurring compounds with anticancer or antiviral properties. In this effort, extracts from a diverse array of organisms collected around the world are tested for biological activity and then fractionated to identify the bioactive compounds. This type of bio-prospecting requires inter-disciplinary collaborations, intuition, and intelligence, but it ultimately requires some serendipity to be successful (http://home.ncifcrf.gov/mtdp/).

Serendipity will always happen, whether we want it to or not and sagacity is needed to make the most of it. Specifically, to maximise serendipitous discoveries requires inter-disciplinary programs, embracing curiosity, an open mind, as well as respect for colleagues. As noted by Louis Pasteur in 1854 "In the fields of observation, chance favours the prepared mind". Discovery is not always driven by market forces. For instance, Viagra® was not the discovery of intentional research, but rather a side effect of a drug tested to treat high blood pressure (http://www.health-viagara.com/viagra-fact.huml). Such accidental discoveries would not have been possible without sagacity (http://newman.baruch.cuny.edu/digital/2001/swazey_reeds_1978/chap_01.htm).

In contrast, serendipity in environmental science may be positive or negative, as appears to be the case with the accidental sinking of tanker cars containing liquid chlorine in near-shore marine waters that took place in British Columbia, in 1975 (O'Connor 1975, MacDonald 1977). To date, those tanks have still not been located and there remains a possibility that a catastrophic breach could, under a worst case scenario result in a large number of human fatalities. Whether serendipity will obviate such a scenario or limit its extent remains to be seen.

A positive example of serendipity in environmental science results from the availability of state-of-the-art instrumentation for chemical investigations, such as the analysis of metals by ICPMS. Traditionally, when asking questions about ecosystem health, a small suite of metals were selected for analysis because these analyses were performed by atomic absorption spectroscopy and were labour intensive. However, with the advent of ICP-MS, concentration data for 50 or more elements are routinely available from many analytical labs. This has sometimes opened the door to unexpected observations, such as anomalous levels of vanadium in sediments from Saint John Harbour, New Brunswick. In this case, the excess vanadium originated from an oil-fired power plant on-shore, and served as a useful tracer for monitoring the dispersal of dredged materials in the harbour (Parsons *et al.* In Press). This is yet another example of being "good" as well as "lucky".

Another positive example results from the availability of statistical software which pointed to unpredicted relationships between environmental variables whether chemical, physical or biological in complex data sets. These data may even have been collected for different purposes. Once the connections were observed, a testing of hypotheses and rational explanations could often result from what would otherwise be hidden in a vast data set or appear as random correlations. There is also serendipity during the interpretation of data, where papers read for no specific purpose can help prove or make a discovery.

We all realise that environmental science is a relatively young field of research that will progress with time and as long as society and governments invest time and money in their responsibility. Sagacity will lead the way to serendipity.

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Best student paper awards/Prix pour les meilleurs exposés par des étudiants

Best Platform Paper Award

Janet McCann, University of Waterloo, Waterloo, ON.
Eisenia fetida or *E. andrei*: which species are you using?

Best Poster Paper Award

Alexa Alexander, University of New Brunswick, Fredericton, NB
Effects of the insecticide imidacloprid on *Epeorus longimanus*
and *Lumbriculus variegatus* feeding and growth.

Other platform paper awards:
2nd (tie). Carrie Rickwood, University of Saskatchewan, Saskatoon, SK; and
Angella Wallace, University of Waterloo, Waterloo, ON.
Honourable mentions: John Dungavell, Queen's University, Kingston, ON; Brian Robinson, Dalhousie University, Halifax, NS; Genevieve Vallieres, University of New Burnswick, St. John, NB; and Patricia Vidella, University of Waterloo, Waterloo, ON.

Other poster paper awards:
2nd. Sarah Stoughton, University of Saskatchewan, Saskatoon, SK.
3rd. Christine Daly, University of Windsor, Windsor, ON.
Honourable mentions: Leanne Baker, University of Windsor, Windsor, ON; Sandra Brasfield, University of New Brunswick, St. John, NB; and Erin Robertson, University of Saskatchewan, Saskatoon, SK.

We wish to acknowledge these individuals who served as judges for these awards:

Sarah Armstrong, Univerity of Saskachewan; Shannon Bard, Dalhousie University; Kent Burnison, Environment Canada; Les Burridge, Department of Fisheries and Oceans; Peter Chapman, EVS Environment Consultants; John Clarke, Environment Canada; Simon Courtenay, Department of Fisheries and Oceans; Paula Dehn, Canisius College; Ken Doe, Environment Canada; Bill Ernst, Environment Canada; Marlene Evans, Environment Canada; Wayne Fairchild, Department of Fisheries and Oceans; Tim Fletcher, Ontario Ministry of Ministry; Chris Fraikin, Golder Associates; Kirk Gustafson, National Cancer Institute; Kathleen Hedley, Environment Canada; Kats Haya, Department of Fisheries and Oceans; Karen Kidd, University of New Brunswick; M. Lebeuf, Department of Fisheries and Oceans; Lucy Lee, University of Waterloo; Deborah MacLatchy, University of New Brunswick; Mary Murdoch, Jacques Whitford Environmental Ltd.; Arthur Niimi, Department of Fisheries and Oceans; Patty Orr, Minnow Environmental Inc.; Jim Sherry, Environment Canada; Murray Smith, Forest Research; Sylvie St. Jean, Environment Canada; Graham van Aggelen, Environment Canada; and Sandra Whiteway, Jacques Whitford Environmental Ltd.

Student interaction is also an important activity at the workshop, we wish to acknowledge these individuals who served as mentors:
Shannon Bard, Dalhousie University; Scott Carr, Texas A&M University; Richard Casey, Alberta Environment; Gordon Craig, G.R. Craig and Associates; Paula Dehn, Canisius College; Monique Dubé, Environment Canada; Marlene Evans, Environment Canada; Mary Murdoch, Jacques Whitford Environmental Ltd.; Marion Nipper, Texas A&M University; Leslie Novak, Stantec Consultants; and Gladys Stephenson, Stantec Consultants.

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The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

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